Importance, Biology, Epidemiology, and Management of Loose Smut (*Ustilago nuda*) of Barley (*Hordeum vulgare*): A Review

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Abstract: Barley (*Hordeum vulgare*) is an economically, nutritionally and industrially important cereal crop worldwide. Ethiopia is believed to be the center of origin and diversity of the cultivated barley crop. The crop has been cultivated in the country since time immemorial. The midlands and highlands of Ethiopia are suitable for barley cultivation. However, there are many biotic and abiotic factors that reduce production and productivity of the crop in Ethiopia and elsewhere. Of the various fungal plant diseases limiting barley productivity, loose smut (*Ustilago nuda*) is one of the major cosmopolitan and destructive seedborne pathogen in many barley-producing countries in the world. This piece of work was undertaken to: 1) review the economic importance and ecological requirements of barley and extent of grain loss due to barley loose smut; 2) review the biology and ecological requirements of the pathogen leading to epidemics; and 3) compile the management options for sustainable barley production and productivity. To achieve these objectives, data and information were gleaned from scientific journal publications, PhD dissertations, Master's theses, research reports, books and book chapters, proceedings and symposia papers, relevant compendia, internet resources, personal communications, and similar other resources. From the reviews made, it could be deduced that barley loose smut on average causes estimated grain yield losses that range from 25 to 30% in the world. The systemic pathogen is embedded in the scutellum part of the embryo and easily transmitted to the next cropping season. The pathogen sori commonly replace the spike during anthesis and healthy ears are infected at flowering through the teliospores blown by wind. Warm soil when seedlings emerge is more conducive to loose smut than cold soil; however, moderate temperature (15 to 22 ^{o}C) and damp cloudy weather or heavy rainfall at flowering time are the preconditions required by the pathogen for heavy infection because of elongated or extended period of open flowers. Barley loose smut can be better managed through the use of proper cultural practices in integration with hot water and solar heat seed treatment, use of resistant varieties, and effective systemic fungicides, like Azoxystrobin, Carboxin, Difenoconazole, Mancozeb, Propiconazole, Tebuconazole, Triadimenol, and Triticonazole. It could, thus, be concluded that barley loose smut is a very important disease that seriously affects barley production and productivity worldwide, but can be reasonably managed through the use of smut-free seed, certified seed, host resistance, and hot water/solar heat or systemic fungicidal seed treatment or their integration.

Keywords: Barley; loose smut; seedborne fungi; seed treatment; *Ustilago nuda*; yield loss.

1. Introduction

According to Vavilov (1951), barley (*Hordeum vulgare* L.) has a diversity of forms and genes and Ethiopia is a center of origin for the crop. New studies supporting the polyphyletic origin of the crop also have indicated Ethiopia as one of the centers of origin of barley (Azeb*et al*., 2016). Barley cultivation probably began in the highlands of Ethiopia and Southeast Asia in prehistoric times. Remains of barley grains found at archaeological sites in the Fertile Crescent indicate that the crop was domesticated 10,000 years ago from its wild relative *Hordeum spontaneum* (Badr *et al*., 2000; Zhou, 2010). Also, the same scholars stated that the wild populations from Israel-Jordan are molecularly more similar than are any others to the cultivated gene pool. Landraces from the Himalayas and India indicate that an allelic substitution has taken place during the migration of barley from the Near East to South Asia and, thus, the Himalayas and India are considered as regions of domesticated barley diversification (Badr *et al*., 2000). Accordingly, after detailed molecular characterization of 317 wild and 57 cultivated barley lines, Badr *et al*. (2000) generally concluded that the Israel-Jordan area in the southern part of the Fertile Crescent has the highest probability of being the geographical area within which wild barley was domesticated; and wild populations found in the southern part of the Fertile Crescent in western Iran have also contributed germplasm to the cultivated barley on its way to the Himalayas.

The barley cultivation time is believed to extend back to 5000 BC in Egypt, 3500 BC in Mesopotamia, 3000 BC in north-western Europe, and 2000 BC in China (Tiwari, 2010; Zhou, 2010). The authors added that barley was the chief bread plant of the Hebrews, Greeks, and Romans and of much of Europe through

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the 16th century. Today barley is cultivated worldwide where the major producing countries are found in the temperate areas and in high elevations of the tropics and subtropics, including African countries, Australia, Canada, China, European Union countries, India, Iran, Russia, Turkey, USA, and others, where China, India, Russia and USA are the major barley producers (Zhou, 2010). Eticha *et al.* (2010) also stated that barley has a long history of cultivation and diverse agro-ecological and cultural practices in Ethiopia.

Barley is known to be an important staple food, industrial crop and animal feed worldwide, ranking fourth after wheat, maize and rice (Asaad *et al.*, 2014). It is a very important grain in the world today and it ranks fourth in both quantity produced and in area of cultivation of cereal crops in the world. The annual world harvest of barley from about 56.52 million hectares in the late century was approximately 140 million tons, with the average yield ranging between 2.50 to 4.00 tons per hectare during the period of spanning from 2001 to 2007 (Tiwari, 2010; Zhou, 2010). The world area under barley cultivation, annual production and productivity for 2016/17 cropping season were 49.28 million hectares, 147.06 million metric tons, 2.98 metric tons per hectare, respectively, while for 2017/18 cropping season the estimates were 47.81 million hectares, 143.68 million tons, and 3.0 metric tons per hectare, respectively(USDA, 2019). Similarly, the world area under barley cultivation, annual production and productivity during the 2018/19 cropping season were 49.23 million hectares, 140.71 million metric tons, and 2.86 metric tons per hectare, respectively (USDA, 2019).

In Ethiopia, barley has been grown as one of the most important staple food crops in the mid-lands and highlands and was cultivated on 44,929.97 hectares of land and produced 110,813.15 tons of grain, with productivity of 2.47 t ha^{-1}, during 2016/2017 main cropping season (CSA, 2017). Similarly, it was cultivated on 951,993.15 hectares of land and produced 2,052,996.372 tons of grain, with 2.16 t ha^{-1}, during 2017/18 main cropping season (CSA, 2018). The crop has a great value in the social and food habits of the Ethiopian people, being used for preparing various types of foodstuffs (*injera* or flattened pancake, bread, porridge, *muq, beso, kinche*, *chiko*, and *qolo*) and local drinks (*tela, borde* and *araqee*) and industrial beverages (beer and malt products) (Amare *et al.*, 2014; Wallelign *et al.*, 2015; MoANR, 2016). Nutritionally, barley is rich in carbohydrates, with moderate amounts of protein, calcium and phosphorus (Zhou, 2010). It is also a source of B vitamins, essential minerals and rich in fiber content, particularly beta-glucan, which has many health benefits (lowering blood sugar and checking cholesterol deposition for safety against heart ailments) (Tiwari, 2010). In Ethiopia, the straw is also used for thatching roofs, house-wall plastering paste mixed with mud, mulching, padding/bedding materials, fuel and animal feed, especially during the dry season (Kuma *et al.*, 2011; Amare *et al.*, 2014). Based on its economic importance today, many barley varieties have been released, of which 37 food and 17 malt barley varieties are under cultivation in Ethiopia, mainly produced in Arsi, Bale and Showa (MoANR, 2016).

Barley productivity in the country, however, is very low (2.47 t ha^{-1}) (CSA, 2017) compared to that of most other countries (3 to 4 t ha^{-1}) (USDA, 2019) due partly to biotic and abiotic factors and other factors influencing yields negatively. Plant diseases, insect pests, weed competition, low-yielding varieties, soil fertility and reaction, climatic factors and poor farming systems are among the most important factors that reduce grain yield and quality of barley in Ethiopia (Amare *et al.*, 2014) and elsewhere (Mathur and Jørgensen, 1992). Today barley crop diseases cause or incur considerable yield and quality losses in Ethiopia (Wallelign *et al.*, 2015).

Although many plant diseases are recorded on barley, scald (*Rhynchosporium secalis*), net blotch (*Pyrenophora teres*), spot blotch (*Cochliobolus sativus),* leaf rust (*Puccinia hordei*), stem rust (*Puccinia graminis* f.sp. *hordei*), smuts (*Ustilago hordei* and *U. nuda*) and eyespot (*Pseudocercosporella herpotrichoides*) remain to be the most widely distributed and economically important diseases of the crop in Ethiopia (Getaneh *et al.*, 1999; BARC, 2000; MARC, 2002; Kiros, 2004; Meki and Asnakech, 2004), of which loose smut (*Ustilago nuda*) is the major among the diseases (Wallelign*et al.*, 2017).

The pathogen is a common cosmopolitan internally seedborne microorganism, whose mycelium is localized within the embryo. It spreads systemically and asymptomatically in the developing plant and the inflorescence is largely replaced during flowering or heading by sori containing teliospores and reducing yield and quality of harvested seeds for next planting (Vánky, 1994). Affected plants in the particular season cannot produce any grain. However, seeds infected by loose smut fungus produce undistinguishable normal and healthy-looking tillers up until the time of ear emergence (Wallelign *et al.*, 2015).

In Ethiopia, most farmers do not know the mechanisms of survival of the pathogen, how it infects the host plant, what factors favor the disease/pathogen and lead to epidemics over time, and how to fight the disease for sustainable barley production and productivity. Exhaustive research information on barley loose smut is also limited for references by stakeholders. Hence, reviewing and compiling pertinent aspects of barley loose smut and the causal pathogenic agent is of paramount importance.

The general aim of this piece of work was to review the published research papers and professional books on barley loose smut and its management options during the past few years with especial reference to

Ethiopia, providing information, research data and knowledge to end users (researchers, students, farming community, policy-makers and other stakeholders).

The review was carried out with the specific objectives to: Discern into the biology and ecological requirements for sustainable barley production; Examine the economic importance of barley loose smut in Ethiopia and elsewhere; Compile the biology of *Ustilago nuda* that causes barley loose smut; Review the environmental factors suitable for *Ustilago nuda* leading to epidemics; and Review the possible management options against barley loose smut.

2. Biology and Ecological Requirements for Barley Production

2.1. Biology of Barley

Barley (*Hordeum vulgare* L. subsp. *vulgare*) belongs to the genus *Hordeum* L. in the tribe Triticeae of the family Poaceae (Gramineae) (Zohary and Hopf, 1993). The genus comprises more than 30 wild grass species distributed in the temperate and arid regions of the world. Barley is most conspicuously characterized by its inflorescence that is a spike instead of the panicle that occurs in most other grasses. The wild progenitor of the cereal is *H. vulgare* subsp. *spontaneum* from Southwest Asia and it is easily crossable with its wild progenitor (forming the primary gene pool of barley). Barley's secondary gene pool is of little value; but the tertiary gene pool holds traits for pathogen resistances and adaptations to extreme environmental conditions, which are of high value if they can be transferred into cultivated barley or other cereals (Blattner, 2018). The greatest food and malt barley diversity in morphological types, genetic races, disease resistant lines, and endemic morphotypes exists in Ethiopia since ancient times (MoANR, 2016).

According to Tiwari (2010), barley plant has several cylindrical culms with hollow internodes separated by solid nodes; and typically with 5-7 internodes on a culm (60–120 cm tall). The single leaves consist of tubular sheath and blade, are borne alternately on opposite sides at each internode, where the leaf sheath encases the culm and extends from the node to which it is attached to almost the whole length of the next internode. At the junction of the sheath and the blade, two colorless or pigmented lateral projections, called 'auricles' or 'claws', are also formed and the leaf-blade is long, flat and narrow with parallel veins. The barley's flower (inflorescence), commonly called 'ear' (spike of spikelets), is distinguishable into two morphological types – six-rowed and two-rowed (Photo 1).

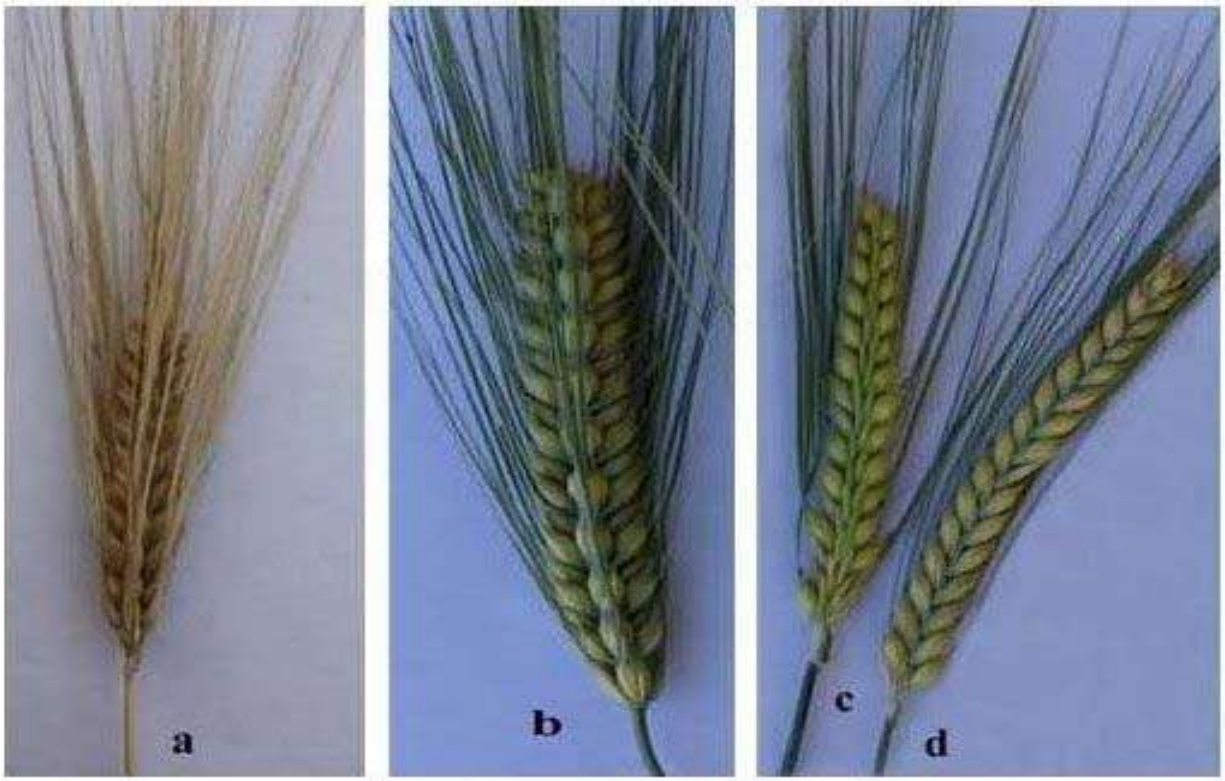

Photo 1. Matured 6-rowed barley ear (a); green 6-rowed ear (b); 2-rowed ear with sterile lateral spikelets (c); and 2-rowed ear with rudimentary lateral spikelets (d). (Tiwari, 2010).

2.2. Ecological Requirements for Barley Production

Barley is a very versatile crop in every way and has been well adapted through its evolution. Much of the world's barley is produced outside of the regions where cereals, such as maize and rice, can grow well, extending into the arctic or subarctic areas (Zhou, 2010). Cultivated barley is grown in a range of diverse environments that vary from subarctic to sub-tropical, with greater concentration in temperate areas and high altitudes of the tropics and subtropics. It is also found in most areas with Mediterranean climate too (Zhou, 2010). Other than the cool highlands, barley is rarely grown in the tropics as it is not suited to warm humid climates (Nevo, 1992). Generally barley has a wider ecological range than any other small cereal crops (Bukantis and Goodman, 1980). Annual rainfall of 190 to 1760 mm, annual temperature ranging from 4.3 to 27.5 °C and soil pH of 4.5 to 8.3 are suitable for barley production and does well on light or sandy loam soils (Duke, 1983).

Barley grows well at altitudes of 1500 to 3500 meters above sea level (m.a.s.l.) and is predominantly grown at 2000 to 3000 m.a.s.l. in Ethiopia (MoA, 1998). In the country, barley is commonly cultivated twice a year, i.e. during *belg* (short rainy period from March to May) and *meher* (long rainy season from June to October). The major barley-producing regions in the country are Amhara, Oromiya, Southern Nations, Nationalities and Peoples' and Tigray Regional States (MoANR, 2016).

2.3. Agronomic Practices and Harvesting

Duke (1983) stated that the seedbed for barley sowing should be prepared to good tilth and the seed is sown by broadcasting or drilling in shallow furrows about 22 cm apart. The recommended depth of sowing is 1.3–4.5 cm. Seeding rates could vary from ca. 65 to 100 kg ha^{-1}. The crop may be raised under both rainfall and irrigated conditions. In dry areas, two to three times watering may be required after sowing. Application of fertilizers containing nitrogen, phosphorus or potash, in various combinations, would influence yield and quality of grain. The recommended blanket fertilizer rates for small cereals in Ethiopia are 46 kg $P_2O_5ha^{-1}$ and 41 kg N ha^{-1} (MoANR, 2016). Additional nitrogen increases yield of straw and grain, but in larger doses, nitrogen increases the protein content and negatively affects its brewing quality. Phosphate fertilizers lower the protein content considerably and influence formation and ripening of grain. Weeding practices are done manually, using mechanical means and/or use of herbicides (commonly selective herbicides). Harvesting and threshing are done manually in developing countries and combine harvesters are employed in developed countries. However, the crop is very prone to smuts under barley production systems of Ethiopia.

3. Economic Importance of Barley Loose Smut

Barley is affected by three smuts, namely covered smut (*Ustilago hordei*), black semi-loose smut (*Ustilago nigra*) and loose smut (*Ustilago nuda*) (Asaad *et al*., 2014). According to these authors, covered smut and black semi-loose smut are due to surface-borne (externally seedborne) pathogens that infect emerging seedlings and develop systemically, while loose smut infects barley during flowering and survives systemically as dormant mycelium in the seed embryo. Loose smut is virtually widely distributed throughout the world and found everywhere in places where barley is grown (Afanasenko *et al*., 2004; Afanasenko, 2009; Johnson, 2014; Zang, 2017).

3.1. Yield and Economic Losses of Barley due to Loose Smut

Root, foliar, and head plant diseases, like smuts, commonly account for yield losses of up to 20-30% (Evans, 1999). Barley loose smut is an internal seedborne disease found wherever barley is grown and is a serious threat to crop yields (Larter and Enns, 1962; Malone and Muskett, 1964) and is due to infection that results in replacement of inflorescence by teliospores of the pathogen (Bailey *et al*., 2003). This monocyclic disease is known to cause crop yield losses, which are approximately equal to the percentage of infected plants within a field but with little or no effect on seed or grain quality (Menzies, 2008; Johnson, 2014). For example, a 5% infection generally leads to a 5% yield reduction; however, occasionally highly susceptible varieties sustain losses in excess of 30% due to the high carry over initial inocula embedded in the embryos (Sherwood, 1997). Grain losses less than 1% are reported in the literature in modern times; but losses of 15 to 25% can occur in the absence of proper management practices (Wallelign *et al*., 2015). The incidence of loose and covered smuts had previously decreased substantially in North America and European countries as a result of use of more effective seed treatment systemic fungicides, and use of more resistant varieties (Zillinsky, 1983). However, barley yield losses of 10 to 30% due to loose smut are still common and encountered in some countries (Zang, 2017). Previously, Bekele *et al.* (1994) reported an incidence of 28% for barley loose smut in Western Amhara, Ethiopia.

Furthermore, a field survey conducted in Awi, South Gondar and West Gojjam Zones of Ethiopia in 2014 indicated a loose smut incidence ranging from 4.04 to 10.64% at field level, whereas seed samples showed a maximum infection of 25.65%, which was actually too high to tolerate (Wallelign *et al*., 2015). Similarly, Tolessa*et al.* (2015) reported a 20% loose smut severity on major cereal crops (including barley) in Borana Zone, Ethiopia. From thisevidence, one can conclude

that barley grain yield loss due to loose smut in Ethiopia ranges from ca 5 to 25%, varying with location, inoculum level in the seed used for planting, the barley variety, weather conditions and level of management practices. If we assume that a hectare of barley yields 3,000 kg grain and if there is 20% loose smut severity, the grain yield loss from the hectare of barley harvested would be (3,000x20)/100 = 600 kg. If the current price of 1 kg grain on market is ETB 20, then the economic or financial loss due to loose smut per hectare would be 600 kg x ETB 20 = ETB 12,000, which is so high that smallholder barley growers cannot afford to tolerate.

3.2. Symptoms and Host Ranges of Barley Loose Smut

Barley loose smut symptoms commonly appear at the flowering stage and become apparent at heading or boot stage (Asresie *et al*., 2015; Davis and Jackson, 2017). The meristematic tissue plays an important role for the passive spread or distribution of the pathogen within the plant by invasion (Koch *et al*., 2013). The symptoms become obvious between heading and maturity and barley heads are initially black to dark brown and some diseased heads may be taller than any of their healthy neighbors. While most affected heads emerge slightly earlier than the normal ones, their spikelets may be entirely transformed into a dry, olive brown teliospore masses in the sori (Photo 2 and 3) (Neate and McMullen, 2005; Afanasenko, 2009; Johnson, 2014; Hills, 2018). Under some environmental conditions, striated sori may also develop on the flag leaves, sheath, and culms of certain varieties (Sherwood, 1997).

Photo 2. Typical symptoms of barley loose smut at flowering stage of the crop, showing rachis covered with sori of the pathogen (Hills, 2018).

Photo 3. Symptoms of barley loose smut at heading (A); with sori completely replaced the ear (B); smutted heads mixed with healthy ears (C); close-up view of healthy and smutted heads (D) (Johnson, 2014; Thomas *et al*., 2017).

Generally, loose smuts are host specific with their own particular *forma specialis* (f.sp.). For example, loose smut of wheat does not infect barley or oats (Thomas *et al*., 2017). The pathogen causing barley loose smut is an obligate monocyclic parasite and attacks cultivated barley and other *Hordeum* species (Neate and McMullen, 2005), wheat, oats, rye, triticale and many other grasses, of course, with the respective *forma specialis* (Menzies, 2008; Menzies and Gaudet, 2009).

4. Biology of *Ustilago nuda*

Thomas *et al.* (2017) stated that infected seed shows no symptoms and appears normal. But when infected seed germinates, the fungus becomes active again and grows slowly in the growing point of the plant. Diseased

plants appear to grow normal but may be slightly taller and earlier maturing than surrounding healthy plants; and at heading, the fungus forms a compact spore mass to replace all florets within the cereal heads.

4.1. Taxonomic Classification of the Pathogen

The pathogen belongs to the class Basidiomycetes, order Ustilaginales, family Ustilaginaceae, genus *Ustilago* and species *Ustilago nuda* (Afanasenko, 2009). Basidiomycetes are characterized by the sexual spores known as teliospores. Upon germination, the teliospores produce four-celled basidia or promycelia. However, unlike the basidia of barley covered smut fungus (*Ustilago hordei*), the basidia of *Ustilago nuda* do not form the sexual spores known as 'basidiospores' or 'sporidia'.*Ustilago nuda* f.sp. *hordei* resembles *Ustilago segetum* f.sp. *segetum* (syn. *Ustilago nuda* f.sp. *tritici*) of loose smut fungus of wheat in almost all important features though it is not as common as loose smut of wheat and covered smut (*Ustilago hordei*) of barley (Malone and Muskett, 1964; Singh, 1982; French and Schultz, 2009; Menzies *et al*., 2014). A number of physiologic races of the fungus are known to occur and some host varieties exhibit a high degree of resistance (Malone and Muskett, 1964).However, two physiological races (a virulent, capable of overcoming the recessive resistance gene present in differential variety, and fungicide-tolerant races) were identified in Ireland in 1984 (Dhitaphichit and Jones, 1991) and existence of some more races are expected in other countries. The authors also reported that several crops grown from seed treated with Vitavax (active ingredient Carboxin), which had been imported from France, contained a Carboxin-tolerant race of *U. nuda.*

The *Ustilago nuda* f.sp. *hordei* fungus produces a hyaline, dikariotic mycelium in host tissue (Sherwood, 1997) and, at maturity, the hyphae of the mycelium thickens and fragments into teliospores (chlamydospores), which are olive brown by transmitted light, paler on one side, sub-spherical or globose, to ovoid, shortly spiny, and covered with very thin membrane and 3.6-10.0 μm (most often 5.5-6.0 μm) in diameter and minutely echinulate (Zillinsky, 1983). The teliospore germinates to form a basidium and compatible basidial cells or short hyphae produced by the former fuse to form infectious dikaryotic mycelium (Sherwood, 1997; Afanasenko, 2009; Asaad *et al*., 2014). It is also noteworthy that *Ustilago nuda* does not form basidiospores (sporidia) during germination on artificial media (Zillinsky, 1983; Sherwood, 1997). Generally, *Ustilago* spp. can be grown on artificial media, but the rate of growth of such colonies is relatively slow and the use of the colony appearance as diagnostic feature in routine seed health testing would be of little or doubtful value (Malone and Muskett, 1964). Thus, the pathogen behaves like obligate microorganism when attempted to cultivate it on artificial media.

Physiologic races of *Ustilago nuda* exist and can hybridize to produce new races, but the biology of the organism makes this a slow process. Host plant resistance is usually conditioned by dominant genes. In some cases, modifier genes may function together with the dominant genes in conditioning resistance (Sherwood, 1997). Unfortunately, some of these genes have been overcome, on a gene-for-gene basis, by recessive genes in the pathogen (Menzies, 2008).However, adequate studies are not made on the presence or absence of physiological races of the pathogen in Ethiopia. Similarly, extensive researches have not been conducted to identify barley resistant/moderately resistant varieties regardless of the presence of several released and cultivated varieties (over 54 cultivars) in this country. Even the presence or absence of the pathogen or extent of seed embryo infection is not easily determined before sowing or planting since such service is not accessible to the farming communities in the country. As a result, comprehensive studies are required to better understand the characteristics of the pathogen and its physiologic races in Ethiopia and elsewhere. Genetic investigations and molecular characterization are appealing for plant breeding activities in the efforts to develop resistant varieties against *U. nuda.*

4.2. Pathogenicity/Pathogenesis of *Ustilago nuda*

Floral infection is initiated by teliospores landing on the open flowers (Zillinsky, 1983). *Ustilago nuda* teliospore germinates and infects developing seed embryo in the host flower and survives to the next host generation as dormant mycelium in the embryo of the seed (Bailey *et al*., 2003; French and Schultz, 2009). Hence, *U. nuda* can be described as an internally seedborne pathogen since it is carried systemically in the infected seed (Zillinsky, 1983; French and Schultz, 2009). When infected seed is sown, the seed germinates and the fungus also grows systemically within the seedling colonizing meristematic tissue; later, the mycelia reach maturity when spikes develop, producing smutted heads (French and Schultz, 2009).

The mycelium of *U. nuda* breaks dormancy when the barley seeds germinate (Menzies *et al*., 2014). That means, when infected seeds germinate, the fungus is stimulated to grow to the growing point and quickly ramifies and moves into the shoot apex, crown node or culm nodes, and seed primordia (French and Schultz, 2009; Johnson, 2014; Menzies *et al*., 2014). As the barley plant grows and eventually 'shoots' to produce its ears, the fungus is carried upwards in the inflorescence that is converted into sori covered with fragile pericarp membranes, which would easily rupture and release the teliospores for spread and next infection (Jones, 1999). The mycelium of *U. nuda* is specifically embedded in

the scutellium tissue of infected seeds, colonizing the embryo largely intercellularly, within the infected plant, being found particularly in the nodes and the ear (Batts and Jeater, 1958). The released teliospores alight on open flowers or developing grains and cause infection by growing through the ovary wall (Sherwood, 1997). In this way, the mycelium,which develops sporiferous hyphae, penetrates all spikelet tissues except the rachis and the awns (Shinohara, 1976; Jones, 1999).

At flowering, the teliospores are blown away by wind from the infected spikes and infect spikes of healthy plants where the spores settle in healthy flowers into which they germinate and infect the embryo at the same time of pollination of the developing grain (Yahyaoui *et al*., 2003; French and Schultz, 2009). Mild temperatures and humid conditions facilitate spore germination and penetration of the ovary by germ tube, developing a mycelium in the embryo (French and Schultz, 2009). The dikaryotic infection hyphae proceed between and through the cells to the developing embryo and become established (Sherwood, 1997; Afanasenko, 2009; Koch *et al*., 2013; Asaad *et al*., 2014). In the meantime, the mycelium remains dormant in the asymptomatic seed until the seed germinates at planting in the next crop season (French and Schultz, 2009). In some barley varieties that exhibit physiological resistance, the fungus may become established in the embryos but diseased plants do not develop (Hewett, 1979).

4.3. Life Cycle/Disease Cycle of *Ustilago nuda*

Barley loose smut has relatively uncomplicated disease cycle and there is no spread between plants during the crop growth period, i.e. it is a monocyclic disease The relationship between seed infection and plant is relatively constant and it is only at flowering stage when seed re-infection takes place that environmental conditions influence disease development (Jones, 1998).

Ustilago nuda attacks barley and wheat without any obvious effect on the vegetative growth (Hewett, 1978). The infected seed develops normally, but contains the fungus as dormant mycelium inside the embryo (Koch *et al*., 2013; Menzies *et al*., 2014). The life cycle of *U. nuda* comprises of primary infection at flowering, survival in the form of spores/mycelium inside the seed, secondary infection in form of systemic infection at seedling stage upon germination and prior to emergence; symptom expression with smutted heads at flowering, releasing the teliospores; and their dissemination by wind (Figure 1) (Yahyauoi *et al*., 2003; Johnson, 2014). The teliospores germinate and the resulting hyphae penetrate the developing seed to complete the life cycle (Menzies and Gaudet, 2009). Upon germination of the teliospores, their long, delicate, infection hyphae enter the young ovary of the flower and grow deeply into embryo of the developing seed, but do not kill it. As the grain matures, the loose smut fungus becomes dormant until the following growing season (Zillinsky, 1983). When the infected barley seed germinates, the fungus grows systemically within the new plant as secondary infection. As the barley plant approaches heading, the mycelium penetrates the head tissues and converts them into masses of teliospores (Zillinsky, 1983). Germinating teliospores produce a four-celled promycelium that, in turn, forms branches. The branches elongate, fuse, and rebranch to form mycelium. However, basidiospores are not produced in *Ustilago nuda* (DCS-UnIll, 1990). Furthermore, the infected seeds cannot be distinguished from healthy seeds by visual inspection only (Jones, 1998).

4.4. Survival and Transmission of the Pathogen

Ustilago nuda survives from one growing season to the next or between crop cycles as a dormant mycelium only in the endosperm and within the embryo of infected barley seeds, and the fungus can survive long-term storage of the seed (French and Schultz, 2009; Koch *et al*., 2013; Asaad *et al*., 2014; Johnson, 2014). During the formation of the sori, the hyphae differentiate and fragment into teliospores. Then the sorus membrane breaks down shortly after the heads emerge and frees the teliospores for dispersal by rainfall splashes, insects, wind or other agents (Afanasenko, 2009; Johnson, 2014; Menzies *et al*., 2014). Menzies *et al*. (2014) also indicated that the teliospores are wind dispersed to infect the florets during flowering in case of loose smut or infest the next generation of barley seed at harvest in case of surface-borne covered or semi-loose smut pathogens. Most inocula for loose smut re-infection probably originate within diseased crops and, given suitable conditions at flowering, the disease tends to multiply over successive generations (Asaad *et al*., 2014).

Several authors indicated that infection can spread between neighboring crops and seeds developing in healthy barley crops could become infected by teliospores released in diseased crops 200 m upwind (French and Schultz, 2009; Asaad *et al*., 2014). However, most infections probably occur within 10 m of heads releasing teliospores (Sherwood, 1997). Infected volunteer barley plants can also act as an important inoculum source for developing seeds (French and Schultz, 2009).

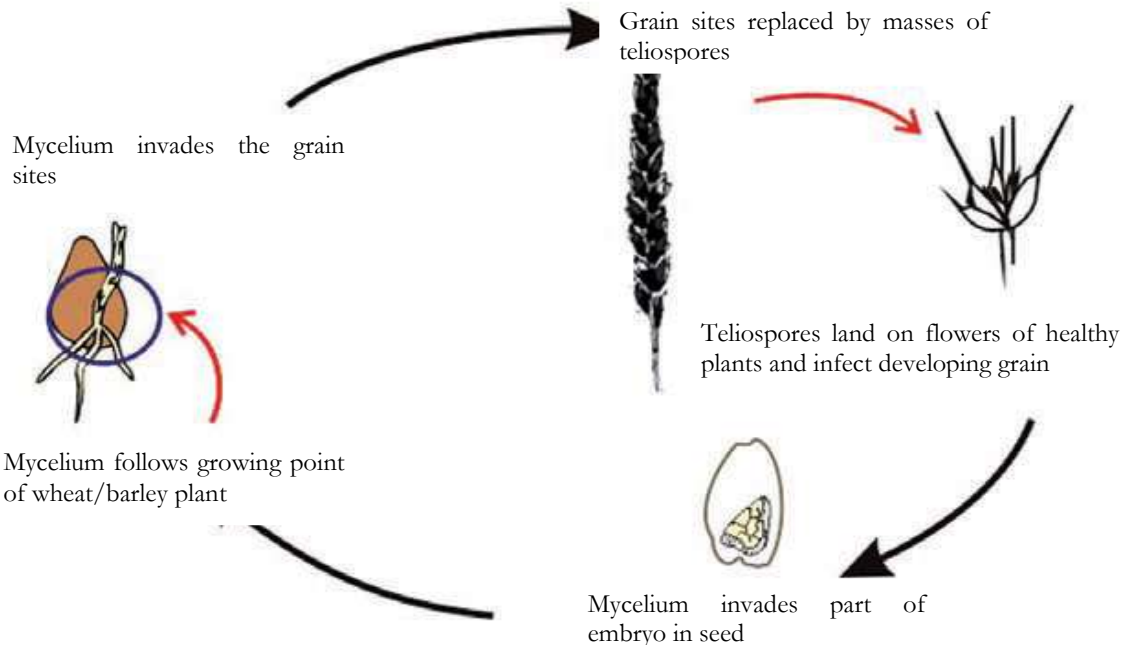

Figure 1. Life cycle of loose smut (*Ustilago nuda*) showing different stages (Kelly *et al*.,2015).

5. Epidemiology of Barley Loose Smut

5.1. Ecological Requirements

According to DCS-UnIll (1990), a warm soil when seedlings emerge seems to be more conducive to loose smut than a cold soil. A heavy infection in the field will often mean a fairly heavy infection in the next crop season. However, even fields with a light infection sometimes produce seed with a high percentage of loose smut. Cool and damp weather at flowering time is necessary for heavy infection because of elongated or extended period of open flowers. Barley loose smut is most common in cool high rainfall areas and may be more common in the year following a wet spring, which promotes seed infection (Asaad *et al*., 2014). Cool, wet, cloudy weather and moderate temperatures (ranging from 15 to 22 ^{o}C) conditions promote longer and more open flowering for the host, allowing more time for teliospores to land on florets and germinate into floral tissue (Sherwood, 1997; Johnson, 2014). Also, a single heavy rain during flowering in affected fields can cause a 10-20-fold increase in infection. Similarly, frequent and high rainfall showers and high humidity at flowering favor infection and lead to development of loose smut epidemics in a susceptible barley variety (Thomas *et al*., 2017). But excessive heat or dry air will lower germination and germ tube growth into the floral tissue, delay penetration of the ovary and preclude the fungus from reaching the growing point (Danko and Michalikova, 1969).

5.2. Assessment of Barley Loose Smut

According to DCS-UnIll (1990), the percentage of loose smut infection in a crop of barley depends on how many of the seeds were initially infected in the field the previous cropping season, which, in turn, is determined by (1) the percentage of infection in the field during the previous season, (2) the weather conditions at the flowering time, and (3) the barley variety. In the field, infection levels are scored as percentage plant infection. Any plant with one or more smutted ears (completely or partially smutted) is recorded as infected (Jones, 1999). A field survey was conducted in five districts of three zones of Western Amhara Region (Ethiopia) in 2014 main cropping season to assess the level of barley loose smut (Wallelign *et al*., 2015). The findings of this study revealed that the incidence ranged from 1.17 to 10.64% and district mean incidence of loose smut varied from 2.91 to 4.52%.

Increasing safe seed movement at the international level necessitates specific solutions to recognize the pathogen at the laboratory level and so reduces the use of fungicide seed treatments and seed health testing is the first step in the pursuit of healthy crops (Asaad *et al*., 2014). Detection of *U. nuda* in barley seed stocks is routinely done by an embryo test method (Malone and Muskett, 1964; Rennie, 1990). The whole embryo count method using staining technique was first applied to cereal loose smut mycelium by Skvortzov (1937) who dissected out the embryos, macerating them in sodium hydroxide and staining with aniline blue (Neergaard, 1979). However, the complete procedures for embryo

count method are outlined by Malone and Muskett (1964), ISTA Handbook No. 25, and ISTA (2014). According to Malone and Muskett (1964) and Neergaard (1979), many workers modified this procedure later, mainly by developing chemical methods for separating the embryos so that many seeds could be tested on a routine basis.

The embryo count method employed by Malone and Muskett (1964) is outlined as follows:

- First 1000 to 4000 seeds (100 – 120 g) are taken and covered with or soaked in 1 L solution of 5 - 10% (usually 5%) sodium hydroxide (NaOH) or concentrated sulphuric acid in a flask, (or placing the seeds in a shallow layer in a large glass dish with 20 cm diameter instead of a flask and using a larger quantity of sodium hydroxide, i.e. 650 mL for 40 g of seed); shaken thoroughly and allowed to remain overnight (24 hours) at 22 ^{o}C to extract the embryos (three replications recommended);
- The contents of the flask are washed on the following day with warm running water or spraying hot water on the mixture through a series of sieves or 10 mesh sieve (hole sizes 3.5, 2.0, and 1.5 mm stacked sieves) to separate the embryos from the glumes and endosperm; (embryos and debris may be further separated after removal from sieves by using funnel containing 50% lactic acid, where the embryos float on the surface and some of the debris sink to the bottom);
- Collecting most of the embryos in the bottom sieve, with a few in the middle one; and placing them in a shallow dish of water to wash the embryos in the beaker and removing the embryos by means of a pipette with a rubber teat; (a small perforated spoon may be used for transferring the embryos instead of using a pipette);
- Placing the embryos in a small beaker and after draining off excess water, adding lactophenol;
- Clearing the embryos by heating on a water bath or Fenwich can or in narrow mouthed bottle using hot water (at 60 – 65 ^{o}C, usually 50 ^{o}C) or by boiling in lactic acid and glycerol (1:2);
- Dehydrating the embryos into a beaker by soaking for 2 min in 95% ethanol;
- Placing them in a 10% KOH and clearing by heating for 5 – 10 minutes;
- Examining the embryos through a stereoscope microscope (25 - 60x magnifications) and compound microscope for checking mycelium using transmitted light. The dark fungus mycelium can be seen in infected embryos without the use of stain;
- The embryos are also examined by placing them in a Petri dish marked with a series of parallel lines 1 cm apart; this method dispenses with the special Perspex tray; and
- Mount embryos in lactophenol and examine using a microscope – bluish mycelium should be visible in the scutellum of infected seed.

N.B. The per cent loose smut in the sample should be calculated based on the number of embryos examined but not on the basis of the number of seeds soaked.

In some areas, tests for embryo infection (including the embryo count method) are used to determine in the laboratory the proportion of infected seed in the seed lots (Sherwood, 1997; Clark and Cockerill, 2011). To detect loose smut (*U. nuda*) infection in cereal seeds requires detailed microscopic examination of at least 1000 individual embryos, which is actually a laborious procedure even with recent refinements, such as the use of sensitive fluorescent stains to visualize the pathogen (Lucas, 1998). The complete procedures for detection of embryo infection by *Ustilago nuda* has been developed and presented by ISTA (2002) and Clark and Cockerill (2011) for end users employing the embryo count method.

Similarly, a new and more rapid detection method for barley loose smut was developed at ICARDA – reducing the test period from two days to just five hours (Asaad *et al*., 2014). The authors outlined that the methodology involves soaking 2000 barley seeds in sodium hydroxide (NaOH) and then heating them at 40 °C for 3.5 hours. This is followed by pre-separation with sodium chloride (NaCl) solution for 15 minutes and then collecting the embryos on 0.71 mm mesh. Afterwards, the embryos are separated using a NaCl solution, with a mixture of 1:1 glycerol and water. The embryos are then checked under the compound microscope for *golden brown mycelium*, which is indicative of infection of the embryo by *Ustilago nuda*. The new method is fast, simple, reliable and very sensitive. The researchers concluded that the test result can be used by seed health laboratories and regulatory and quarantine authorities to ensure that only loose smut-free seeds are introduced (Assad *et al*., 2014).

The new and fast method developed by ICARDA for *U. nuda* detection clearly shows infected embryos in infected barley seeds (Asaad *et al*., 2014). These researchers disclosed that the new method is fast, inexpensive, healthy (no harmful chemicals are used, nor a fume hood), simple, reliable and very sensitive; and they confirmed that results obtained are highly practical, accurate and cost effective, and will facilitate a quick judgment on the presence of *U. nuda* in infected seeds. This tool can rapidly detect the presence of *U. nuda* and is an advantage for routine seed health testing laboratories that conduct tests on large numbers of samples. The scholars recommended that this new method be used in seed health laboratories for research

and quarantine purposes to ensure that only seeds free of loose smut are introduced and planted, and it will play an important role in restricting the spread of this disease via infected seeds. Practically, a laboratory seed health test was conducted using farmers' saved local barley variety seeds (both two- and four-rowed seeds) collected from 15 Farmers' Associations in Western Amhara Region (Ethiopia) in 2014 with the specific objective to determine the infection levels using the embryo count method employed by ISTA (2014). The results of the laboratory test indicated that 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 from the study areas for embryo examination (Table 1) (Wallelign *et al*., 2015). In this respect, the researchers mentioned that the local barley varieties, namely *Awuragebis* and *Semerieta,* were more dominantly infected than the other local varieties. However, when the results were compared with the field survey results, the laboratory results (loose smut infections) were found to be higher than the field survey results.

The development of an effective, rapid and accurate method for detecting the pathogen is advantageous for rapid decision-making at seed health laboratories and quarantine centers and for minimizing the spread of loose smut (Asaad *et al*., 2014). In the 1980s, new diagnostic technologies based on serological characters became available in plant pathology and have been successfully applied to seed testing (Asaad *et al*., 2014). Additionally, during the last 15 years, new techniques have been developed for detecting microorganisms in seeds, based on DNA analysis (Hollomon, 1998; Asaad *et al*., 2014).

Barley loose smut is managed mainly by using healthy seeds or seeds coated with systemic fungicides; however, seed treatment is not recommended in many countries when the threshold is not exceeded, i.e. beyond 5% infection (Asaad *et al*., 2014). Moreover, the authors stated that because seed infected by loose smut can germinate and there are no visible signs to alert users to the pathogen, if the seed is not tested there is a high chance of introducing infected seeds into farmers' fields or new areas. This implies that seed health testing prior to planting is necessary in areas where loose smut is a threat for barley and wheat production.

5.3. Barley Loose Smut Monitoring and Forecasting

Disease monitoring provides information that lays the basis for forecasting system. In barley loose smut monitoring, it enables to inspect the presence or absence of the disease under field conditions, allowing rogueing measures if smutted spikes are detected early in the growth period. Similarly, it ensures freedom of the seed from loose smut for next season sowing.

Table 1. Welch's variance-weighted ANOVA for embryo test on barley seed infection with loose smut from five districts of Western Amhara Region, Ethiopia, during 2014 main cropping season.

District	Location/Farmers' Association	Barley variety	Mean infection (%)	Standard deviation
Farta	Kimirdengia	Awuragebis	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	Titramichael	Semerieta	12.00 (1.14)	0.039
Lay Gayint	Chekoho	Awuragebis	13.00 (1.18)	0.016
Lay Gayint	Genboche	Awuragebis	13.00 (1.18)	0.036
Lay Gayint	Govgov	Tikurdiribgebis	11.95 (1.10)	0.013
Lay Gayint	Sali	Awuragebis	16.55 (1.29)	0.064
Sekela	Ambisi	Wonteka	20.96 (1.34)	0.006
Sekela	Gindatemam	Semerieta	9.90 (0.97)	0.098
Sekela	Ateta	Wonteka	15.05 (1.25)	0.063
YilmanaDensa	Aybar	Semerieta	11.25 (1.11)	0.028
YilmanaDensa	Debremawi	Semerieta	21.40 (1.36)	0.008
Farta	Tsegur	Tsebel	11.00 (1.10)	0.032
GuagusaShikudad	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; and values in parentheses are log transformed data (Wallelign *et al*., 2015).

For barley loose smut diagnosis, it has been suggested to look for scattered plants with black heads or bare flower stalks unlike the covered smut; detection of slightly taller and earlier maturing than the heads of the surrounding healthy plants; having insight into compact masses of dark brown-black powdery spores at heading florets and remnant bare stalks once fungal spores have been blown away (Jayasena and Thomas, 2015). In this connection, one can identify the susceptible varieties from the resistant varieties in the monitoring process in the field. The percentage of smutted spikes in the plant stands in a particular season can be calculated during the monitoring process and field trials too (Neergaard, 1979).

The loose smut monitoring actually deals with detection of the percentage of embryo infection of seeds by *U. nuda* using an easy, rapid and precise routine testing method(s) (ISTA, 2016). This information is helpful for decision-making with reference to the need for seed treatment. Similarly, knowledge of the soil moisture status at planting and the weather conditions at flowering helps for prognostication of loose smut for rogueing purpose. Information on the genetics of the barley variety during monitoring is also desirable for prior action (Borgen, 2004; Menzies *et al*., 2009; Menzies *et al*., 2010). Generally, the disease assessment and prediction or forecasting process has been illustrated diagrammatically (Figure 2) (Lucas, 1998).

5.4. Use of Information Technology in Barley Loose Smut Warning System

In barley loose smut management, information technology can help in providing available information on the nature of the pathogen, how it causes the disease and the ecological conditions that lead to epidemics. Animation of the processes of pathogenesis is useful for training farmers on the pathogen and knowledge on the disease itself. Similarly, it can assist in public awareness creation on availability of the resistant varieties, and sorting susceptible varieties. The information on the loose smut management options is another area where information technology can help the farming communities, particularly in the use of integrated loose smut management by combining the efficient, economical and feasible management tactics. Updating users on the latest technologies with reference to newly developed and released varieties, new systemic fungicides as well as new approaches on the detection of the systemic pathogen in the embryo. Estimation of grain losses due to loose smut and modeling is also desirable.

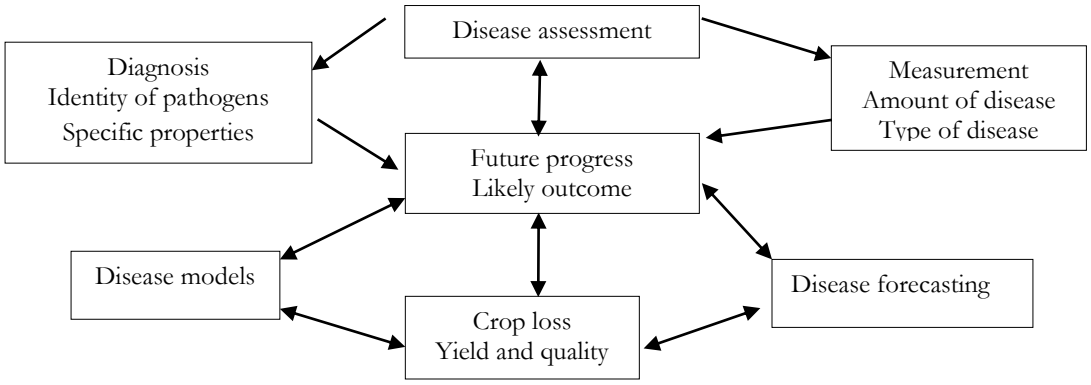

Figure 2. Activities involved in disease assessment and prediction (Lucas, 1998).

6. Barley Loose Smut Management Options

Barley loose smut can be well managed through the use of smut-free seed, certified seed, host resistance and hot water/solar heat or systemic fungicidal seed treatment or their integration (Bailey *et al*., 2003; Menzies, 2008). Management measures can reduce yield losses occurring from loose smut even in areas where development of the pathogen is favored by the environmental conditions and susceptible varieties (Sherwood, 1997).

6.1. Cultural Practices

The cultural practices against loose smut include use of high quality and healthy clean seed produced in smut-free area, hot water treatment, irrigation just after planting to lower soil temperature and allow faster seed germination and emergence, rotating barley varieties from season to season, at least after three to four consecutive years, using optimum isolation distance and rogueing smutted spikes as soon as observed (Evans, 1999; French and Schultz, 2009; Wallelign *et al*., 2015). Growing barley continuously on the same plot results in significantly reduced yields and lower quality grain, especially in the wetter, higher yielding areas (Evans, 1999). The author suggested that producers growing barley on the same farmland season after season or using short crop rotations will need to pay special attention to soil fertility, resistant barley varieties and systemic fungicidal disease suppression either by seed treatment or fungicidal sprays. In Ethiopia, the

traditional methods to manage loose smut of barley is use of crop rotation and rogueing smutted ears; but rogueing may not give satisfactory results because the inocula might have already been blown by wind and contaminated the ovaries.

Hot water treatment at 50 ^{o}C for 10 minutes can kill the internal pathogen embedded in the embryo without harming the embryo (Zillinsky, 1983), while satisfactory results have also been claimed for cold water and anaerobic treatments (Malone and Muskett, 1964). Anaerobic seed treatment using air-tight storages were also used as physical management (Bilgrami and Dube, 2001). According to Chaube and Singh (2001), solar heat treatment effectively controls loose smut of wheat (*U. segetum*), where the seed is soaked in water for 4 hours on a bright day after which the seed is dried in the sun for 4 hours. They also stated that *U. nuda* has been effectively subdued by solar heat. In a solar heat treatment of seed on concrete floor, brown paper and mixing seed with sand at 50:50 ratio for different duration (0, 4, 8 and 12 hours) revealed that seeds dried with sand mixture effectively suppressed all six seedborne fungi (*Alternaria tenuis, Asperillus* spp*., Bipolaris sorokiniana, Curvularia lunata, Fusarium* and *Penicillium* spp.) of wheat in 8 and 12 hours solar treatment (Khan *et al*., 2002).

On one hand, lack of awareness among the farming communities about the nature of the pathogen and loose smut may make implementation of the abovementioned cultural practices difficult. Farmers do not know whether the seed is disease-free or certified. The use of hot water treatment requires skill of measuring the temperature; and they do not also know the value of solar heat treatment. On the other hand, lack of adequate farmland may not allow crop rotation to be practiced in most cases. To minimize such challenges, proper training of farmers on the nature of the pathogen and the disease and cultural management options is advisable. In Ethiopia, the role of development agents, district experts, concerned units and officials is immense in alleviating the problem jointly.

6.2. Host Plant Resistance

The most economical and environmentally benign way of managing barley loose smut is the use of resistant varieties (Menzies *et al*., 2009; Menzies *et al*., 2010). Hence, the choice of resistant varieties is an important component of preventative strategy (Zillinsky, 1983). Resistant varieties have been developed for areas where barley loose smut is a production constraint (French and Schultz, 2009; Menzies *et al*., 2010). Barley loose smut is known to be effectively suppressed by the *Un8* resistance gene isolated by map-based cloning and delimited on chromosome arm 1HL too (Zang, 2017). The scholar also reported that sequence analysis identified a *Un8* candidate gene predicted to be a putative protein kinase with two kinase domains. Some barley varieties display a closed flowering habit and so avoid infection as a means of defense even during years of high loose smut infection (Rennie and Seaton, 1975; Jones, 1998).

Genetic studies have revealed that resistance to *U. nuda* is generally conferred by single, dominant, independently inherited genes (Metcalfe, 1966). However, the incorporation of loose smut resistance genes into new barley varieties can be an arduous procedure because of the time and labor required for testing barley lines for resistance. Moreover, none of the recommended barley varieties are completely resistant to all the physiologic races of the three smut fungi, namely *U. nuda*, *U. nigra* and *U. hordei* (DCS-UnIll, 1990). In this connection, it is commendable if reconnaissance surveys are made across the major barley-producing regions of Ethiopia to check whether or not physiological races of the pathogen are present. It is well known that several barley varieties are developed based mainly on their high yielding performance, released and are under cultivation in Ethiopia (MoANR, 2016). However, their genetic resistance potentials against loose smut are not fully evaluated under artificial inoculation with virulent pathogen isolates, implying the need for future researches for their resistance reaction studies as best option for management strategy to sustain barley production and productivity.

6.3. Biological Control

Wheat loose smut (*U. segetum* var.*tritici*) was suppressed almost completely through seed treatment with any of the bioagents, such as *Trichoderma viride, T. harzianum; Pseudomonas fluorescens* and *Gliocladium virens*in combination with the systemic fungicide Vitavax@ 0.125% (Singh and Maheshwari, 2001). These researchers found that values of smut management were even better than full dose of Vitavax (0.25%). Seed germination percentage in the laboratory, seedling emergence in the field and seed yield per plot were significantly high without any negative effects on the roots or shoots. Since the above causal agent is very similar to *U. nuda* of barley loose smut, the bioagents could be applied against barley loose smut as well. However, *in vivo* test is required before formulation and mass multiplication of any of the bioagents against loose smut locally. Also, there is a need for isolation, identification and characterization of additional indigenous bioagents. Furthermore, introduction of exotic bioagents and testing for their efficacies and adaptation for integration with other management options is an attractive issue and could be an effective strategy for future researches in Ethiopia.

6.4. Chemical Protection

Seed treatment with systemic fungicides usually reduces or eliminates the internally seedborne inoculum very effectively, giving 60-100% suppression of the pathogen (Neate and McMullen, 2005; Clark and Cockerill, 2011; Hills, 2018). Barley seed treatment by applying systemic fungicides or fungicide mixture, such as Carboxin and Triadimenol, would help suppress or reduce all the three loose smut levels, if applied properly (French and Schultz, 2009). However, *U. nuda* is not managed by surface-active protectant or contact fungicides used as seed treatment and, to this effect, management depended on hot water or cold water treatments, which killed mycelium in the embryo without killing the embryo, of foundation and certified seed lots until the 1970s (Sherwood, 1997). The methods could be still used in areas where systemic fungicide seed treatments are not available or accessible.

Tisdale *et al.* (1923) reported for the first time that formaldehyde was as effective as hot water in the suppression of loose smut in six varieties of winter barley. In most parts of the world, correct seed treatment (pickling) with systemic fungicides have been effectively and economically employed against barley loose smut (Thomas *et al.*, 2017). The introduction of Carboxin as a first systemic seed treatment fungicide is a breakthrough though it is too expensive (Menzies, 2008). As treated seed germinates, Carboxin moves into the seedling and either kills or stops the growth of the fungus; however, *U. nuda* isolates that are fully resistant to Carboxin have been detected in Europe (Newcombe and Thomas, 1991; Sherwood, 1997; Menzies, 2008). To avoid the loss of efficacy of systemic fungicide seed treatments, a fungicide rotation scheme in which Carboxin seed treatments are used with demethylation inhibitor seed treatments from year to year would be highly recommended (Menzies, 2008). Similarly, Tebuconazole (Folicur) has recently been registered for use as a seed treatment fungicide on barley and is very effective in managing loose smut (Sherwood, 1997). Currently, there are other fungicide-active ingredients that can be used as seed treatments to manage *U. nuda* on barley and *U. tritici* on wheat and they are triazole type chemicals, namely Difenoconazole, Propiconazole, Tebuconazole, Triadimenol and Triticonazole belonging to the ergosterol demethylation inhibitors (Menzies, 2008).

Hewitt (1998) listed the following chemicals as effective systemic fungicides against loose smuts: Quintozene (1930), Carboxin (1966), Oxycarboxin (1966), Benomyl (1968), Fenfuran (1974), Nuarimol (1976), Triadimenol (1978), Bitertanol (1979), Triflumizole (1982), Diniconazole (1983), Flutriafol (1983), Tebuconazole (1986), Fenbuconazole (1988), Triaconazole (1988) and Epoxiconazole (1990). Similarly, Fuentes-Dávila*et al.* (2002) presented the following fungicides, including Benomyl, Carbathiin, Carboxin, Difenoconazole, Etaconazole, Ethyltrianol, Flutriafol, Furmecyclox, Myclobutanil, Nuarimol and Triadimenol as efficacious against loose smut. Furthermore, foliar application of a number of broad-spectrum systemic fungicides (particularly conazole fungicides) to loose smut-infected plants of wheat and barley in a three-spray program resulted in a marked reduction in the percentage of plants producing infected ears in Ireland (Figure 3) (Jones, 1999).

A barley seed treatment experiment was conducted in Ethiopia with the objective to examine the effects of four fungicidal seed-dressings (Thiram, Apron Star, Dynamic, and Propiconazole) and two coating materials (Genus Coat™ and Disco AG Blue L-237) on loose smut levels (Wallelign *et al.*, 2017). The research results revealed that the seed treatment had highly significant effect on days to emergence and flowering, tiller number, grain yield (maximum 1727.8 kg ha^{-1} with Thiram seed treatment and Genius plus Disco coatings) and smut incidence, where the minimum incidence (0.00%) was recorded from plots sown with seeds treated with Propiconazole, while the maximum (15.83%) incidence was recorded from plots sown with untreated barley seeds (Wallelign *et al.*, 2017). Similarly, the maximum relative efficacies of treatments on the management of loose smut were achieved on plots that received seeds treated with Propiconazole for both coated and uncoated seeds (95.81 to 100%), followed by 83.31 and 75.00% for seeds treated with Thiram coated with Disco plus Genius coat and Thiram alone, respectively. The minimum (29.19 and 33.31%) efficacy was recorded on plots sown with seeds coated with Disco and Genius coats, respectively, without fungicide treatment.

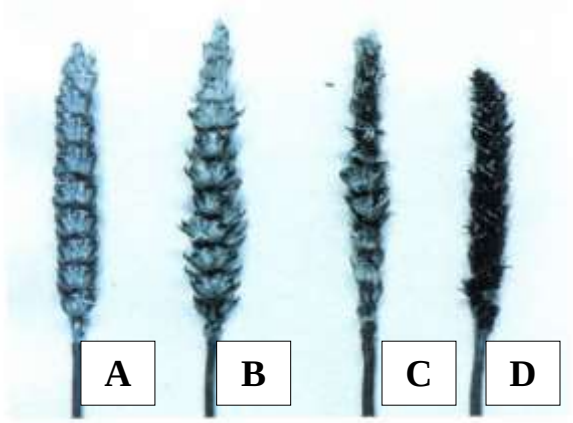

Figure 3. Ears of cv. 'Chinese Spring' from plants grown from loose smut-infected seed. From the left: (A) Healthy ear; (B) ear from plant sprayed with Triadimefon at GS 39 (no signs of infection but lax habit); (C) ear from plant sprayed with Triadimefon at GS 39 (partially smutted ear); and (D) completely smutted ear (Jones, 1999).

Concerning the effects of seed treatment on yield and relative yield losses, Wallelign *et al.* (2017) found that the relative seed yield losses were reduced by all combinations of fungicides with coating materials except Propiconazole. The maximum (51.18%) yield loss was obtained from plots sown with seeds treated with Propiconazole + Disco + Genius coats, while the minimum (2.39%) seed yield loss was obtained from plots sown with seeds treated with Thiram + Disco coat, followed by plots sown with seeds treated with Apron Star + Disco + Genius coat, seeds treated with Dynamic + Disco + Genius coat, and seeds treated with Apron Star alone with corresponding yield losses of 9.02, 8.58 and 9.08% as compared with the untreated check, which had a 21.69% relative yield loss.

6.5. Integrated Barley Loose Smut Management

The use of a single disease control tactic would not bring a desired loose smut management to sustain barley production and productivity. For instance, cultural practices do not completely suppress the disease. Similarly, resistant barley varieties may not be available to the growers or more virulent *U. nuda* strains may appear. Also, use of biological control alone may not give satisfactory results and growers may not have the know-how and the bioagnts themselves. On the other hand, application of fungicides as seed treatment or spray has its limitations. Hence, under severe infection by loose smut, combination of two, three or more measures based on efficacy, efficiency, environment-friendly and affordability would be necessary (Yahyaoui*et al.*, 2003). Similarly, Wallelign*et al.* (2015) suggested selecting and employing disease-free barley seeds and screening resistant/tolerant varieties and integrating them together with seed treatments by effective systemic fungicides to manage loose smut and to sustain barley production and productivity. Use of disease-free resistant/moderately resistant varieties accompanied by seed treatment with hot water/solar heat and/or systemic fungicides (like Carboxin, Difenoconazole, Propiconazole, Tebuconazole, Triadimenol or Thiram) (Davis and Jackson, 2017) would give satisfactory results in barley production. Singh and Maheshwari (2001) also demonstrated in their seed treatment trial that the use of *Trichoderma viride, T. harzianum; Pseudomonas fluorescens* and *Gliocladium virens*in integration with the systemic fungicide Vitavax@0.125% (Carboxin) was effective against smut fungi.

7. Discussion

Since its domestication, barley has been cultivated in the world (African countries, Australia, Canada, China, European Union countries, India, Iran, Russia, Turkey, USA and others), providing food for human consumption, raw materials for industries and animal feed (Zhou, 2010). Also, it has a long history of cultivation in diverse agro-ecologies, especially in the mid-lands and highlands, and is one of the most important staple cereal crops in the country (Eticha *et al.,* 2010). However, the production and productivity of this valuable barley has been constrained by biotic and abiotic factors wherever barley is cultivated. One of the production-limiting biotic factors in Ethiopia and elsewhere is barley loose smut caused by the fungus *Ustilago nuda*.

Sherwood (1997) reported that barley loose smut causes yield losses in excess of 30% on highly susceptible varieties. Bekele *et al.* (1994) previously reported an incidence of 28% for barley loose smut in Ethiopia. Recently, Wallelign *et al.* (2015) reported grain yield losses of 15 to 25% in the same country in the absence of proper management practices. Similarly, Tolessa *et al.* (2015) reported a 20% loose smut severity on major cereal crops (including barley) in Borana Zone, Ethiopia. Zang (2017) also reported that barley yield losses of 10 to 30% due to loose smut are still common and encountered in some countries. This variation in yield losses inflicted on barley due to loose smut could be attributed to the initial seed source, freedom from the seedborne pathogen (sanitary measures), percent initial seed infection, seed treatment practices employed, the weather conditions in the agro-ecologies during flowering, degree of susceptibility/resistance of the varieties used, and seed treatment practices applied before planting.

Barley loose smut symptoms commonly become evident at the flowering stage and become apparent at heading (Asresie *et al.*, 2015; Davis and Jackson, 2017). Barley loose smut may be confused with barley covered smut (*Ustilago hordei*), under field condition because both are seedborne and are similar in their disease cycle (both are systemic pathogens during the vegetative stage), seedling infection processes during seed germination, vegetative growth stage and in the replacement of the spikelets (Koch *et al.*, 2013). However, the life cycles of the two pathogens are different in that *Ustilagohordei* is externally seedborne, while *Ustilago nuda* is internally seedborne pathogen (embedded in the embryo). The sori of *Ustilagohordei* remain intact on the rachis and only break during harvesting and threshing contaminating the healthy seed surfaces. Unlike barley loose smut, the sori due to *Ustilagohordei* are easily distinguished visually from the healthy seeds if they are not broken during threshing. Also, during harvesting by combiner, smoke of teliospores would be released into the sky. On the other hand, the spikelets infected by *Ustilago nuda* would be entirely transformed into a dry, olive brown teliospore masses in the sori (Neate and McMullen, 2005; Afanasenko, 2009; Johnson, 2014; Hills, 2018). The sori due to *Ustilago nuda* are fragile and easily rapture, releasing the teliospores and would be blown to infect open barley flower stigmas and ovules.

Barley loose smut (*Ustilago nuda*) is a monocyclic disease and the causal pathogen behaves like obligate microorganism. The pathogen attacks cultivated barley and other *Hordeum* species (Neate and McMullen, 2005), including wheat, oats, rye, triticale and many other grasses with the respective *forma specialis* (Menzies, 2008; Menzies and Gaudet, 2009). Unlike non-obligate parasites, *Ustilago nuda* can not be cultivated on culture media to produce colonies. The disease is claimed to be monocyclic because multiple generations can not develop in the same growing season although ovules are infected. Of course, the pathogen population can increase over seasons if same infected seeds are sown season after season without change or without seed treatment of any kind. There must be intervention here to provide farmers with disease-free seed of resistant/tolerant varieties and the culture of seed treatment with effective systemic seed treatment fungicides or integrated management options should be developed. Similarly, the detection methods, like the embryo count method, for testing the presence or absence of *Ustilago nuda* in the embryos of seed to be planted demand professional inputs or expertise.

Malone and Muskett (1964) previously reported the existence of a number of physiological races of the fungus and presence of high degree of resistance in some host varieties. However, Dhitaphichit and Jones (1991) reported two physiological races (a virulent, capable of overcoming the recessive resistance gene present in differential variety, and the emergence of fungicide-tolerant races in Ireland and expectation of some more physiologic races in other countries, e.g. seed from France contained Carboxin-tolerant race of *U. nuda*. In Ethiopia, the main hosts of the pathogen are barley and wheat. But the physiological races present in the country are not tangibly characterized and development of resistance to systemic seed fungicides is not tested. This requires due attention from plant breeders and plant pathologists to act accordingly on the issues.

Knowing the preconditions to the establishment and development of any plant disease is decisive to develop apt and viable strategies for its sustainable management options. In this connection, cool and damp/wet weather at flowering time is necessary for heavy infection by *Ustilago nuda* since such weather conditions are known to elongate or extend open flower period. Similarly, loose smut is predominant in high rainfall areas, which promote floral or seed infection (Asaad *et al*., 2014). In other words, cool, wet, cloudy weather and moderate temperatures (15 to 22 ^{o}C) are known to promote longer and more open flowering for the host, allowing more time for teliospores to land on florets and germinate into floral tissue (Sherwood, 1997; Johnson, 2014). These set of conditions are familiar in the medium and highland elevations where barley is principally grown in Ethiopia. It implies that integrated barley loose smut management options should be applied to minimize the yield losses in these specific agro-ecologies. Of course, there must be continuous and appropriate monitoring of the development of the disease from flowering to heading stage over crop seasons to assess the trend of loose smut increase for decision-making.

Cultural practices that include high quality clean and healthy seed, hot water treatment, irrigation just after planting, crop rotation, optimum isolation distance and rogueing smutted heads as soon as observed are suggested against loose smut (Evans, 1999; French and Schultz, 2009; Wallelign *et al*., 2015). The major problem here is lack of knowledge on the nature of the pathogen, its transmission and know-how of application of some methods, like hot water treatment, detection technique of the pathogen in the seed and lack of access to healthy seeds. For instance, seed treatment with hot water at 50 ^{o}C for 10 minutes is suggested to kill the internal pathogen embedded in the embryo without harming the embryo (Zillinsky, 1983). But the local farmers do not have the know-how and facilities for this seed treatment application This requires research calibration, professional efforts and extension services to the farming communities.

According to Menzies *et al*. (2009) and Menzies *et al*. (2010), the most economical and environmentally benign way of managing barley loose smut is the use of resistant varieties. Here the main challenge to the local farmers is that though a number of barley varieties are released, the reaction of these varieties is not well investigated via artificial inoculation with virulent isolates collected from all over the country. Similarly, the physiological races of the pathogen that exist in the country are not tangibly known. After all, the farmers may not have access to the improved varieties and commonly use their own saved seeds that may have carryover embryo-embedded pathogen. All these situations imply that plant breeders, plant pathologists, the extension wing and concerned institutions should work together to provide practical solutions to problems associated with use of resistant varieties.

Singh and Maheshwari (2001) reported that seed treatment with any of the bioagents, including *Trichoderma viride, T. harzianum; Pseudomonas fluorescens* and *Gliocladium virens*in combination with the systemic fungicide Vitavax@ 0.125% almost completely suppressed wheat loose smut (*U. segetum* var. *tritici*), the pathogen that is very similar to *Usilago nuda*. Botanicals may be considered in the same category. The question here is the feasibility issue since these bioagents are under investigation, especially in Ethiopia the biological control strategy is at an infant stage. Isolation and characterization of indigenous bioagents is required; similarly, the adaptability and efficacies of such bioagents should be duly tested and formulation and

mass multiplication of the bioagents should be determined ahead of recommendation.

Several scholars believe that seed treatment with systemic fungicides eliminates the internally seedborne inoculum very effectively, giving 60-100% suppression of the pathogen (Neate and McMullen, 2005; Clark and Cockerill, 2011; Hills, 2018). Menzies (2008) reported that Difenoconazole, Propiconazole, Tebuconazole, Triadimenol and Triticonazole effectively managed loose smut of both barley and wheat. Similarly, French and Schultz (2009) stated that seed treatment with systemic fungicides, such as Carboxin and Triadimenol, would generally suppress loose smut levels, if applied properly. However, several other authors (Newcombe and Thomas, 1991; Sherwood, 1997) and Menzies (2008) also reported that *U. nuda* isolates developed full resistance to Carboxin in Europe. That means, the loose smut causing pathogen becomes insensitive to the systemic fungicide. This implies that there is variability in the pathogen and/or emergence of new physiological race(s) that sharply reduce(s) the efficacy of the seed treatment fungicide. Besides, systemic seed treatment fungicides are expensive and use of fungicides is not environment-friendly. Not only that, systemic seed treatment fungicides are not easily accessible to the local farmers and farmers may not have the know-how of application of such fungicides.

Generally, the use of separate disease control tactics would not give satisfactory and sustainable loose smut management. Hence, it can be comfortably concluded that use of combination of disease-free seed, hot water seed treatment, employment of resistant/tolerant varieties and seed treatment with effective systemic fungicides prior to sowing would alleviate barley loose smut problem wherever the disease is a pressing constraint.

8. Conclusions

Barley loose smut, which is caused by an internal seedborne fungus *Ustilago nuda*, is one of the major diseases of the crop worldwide. In this piece of work, attempts have been made to review the economic importance of loose smut, biology of the pathogen, ecological requirements for epidemics, and management options for sustainable barley production. Barley loose smut could cause crop yield losses of up to 30% or more worldwide. Some studies conducted on barley loose smut in Ethiopia also estimated similar yield losses, especially on susceptible varieties under favorable environmental conditions to the disease and that lead to epidemics. The newly infected and harvested seed externally looks normal and the food quality is not affected, but is not suitable for planting without seed treatment in the next crop season since the pathogen is embedded in it.

There is no single satisfactory method to control barley loose smut worldwide. Resistant or tolerant varieties may not be available or may not have durable resistance. It is known that the variability of *Ustilago nuda* and existence of many physiological races or emergence of new races lead to development of resistance to the effective systemic seed treatment fungicides, like Carboxin. Biological control option is still at an infant stage under research consideration. In Ethiopia, smart farmers try to rogue the smutted barley spikes or heads as soon as observed; but the majority of the local farmers use their own saved seeds since they do not have access to systemic seed treatment fungicides and improved seeds. The implication is to use integrated barley loose smut management option that comprises cultural practices, such as disease-free seeds, use of hot water seed treatment, use of seeds of resistant/tolerant varieties, biological control (even botanicals) and systemic seed treatment fungicides.

It is known that several barley varieties have been released for cultivation in Ethiopia. Besides, there are some landraces in the country. In this regard, plant breeders need to test these materials for resistance to loose smut. It is also desirable to collect the physiological race(s) of *Ustilago nuda* and characterize them using the conventional methods and employing molecular techniques. Screening systemic seed treatment fungicides and calibrating the doses is recommended for practical purpose. Similarly, the use of hot water treatment and/or heating by direct sunlight radiation needs investigation in controlling loose smut. For Ethiopian farmers, the best management options are integrating resistant barley varieties with cultural practices, like use of disease-free seed, timely rogueing smutted spikes at flowering, and seed treatment with hot water as well as effective systemic fungicides. Overall, research on integrated barley loose smut management is timely for sustainable barley production and productivity in the country.

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