Detection of Barberry Plants (*Berberis holstii*) as an Alternate Host of Stem Rust (*Puccinia graminis*) of Wheat in Ethiopia

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Abstract

Berberis is a genus of many species of deciduous and evergreen shrubs. Two species of *Berberis* are present in Africa, *Berberis vulgaris* in northwest Africa and *B. holstii* in the mountains of eastern and southern Africa. The presence of *B. holstii* in Ethiopia is reported in a book of Ethiopian and Eritrean flora; however, there is no information indicating that the plant serves as an alternate host of wheat stem rust (*Puccinia graminis*). Since 2009, Ambo Plant Protection Research Center has initiated studies on distribution of Barberry shrubs in the northern part of the country and collection of aeciospores from the shrub and inoculation of different small cereal crops to confirm whether the aeciospores are function stem rust or not.To meet these objectives, surveys were carried out in the northern part of Ethiopia and identified 19 localities where the shrub is grown. Of these, 12 were in north Shewa, three in Wello zones of Amhara region and four in south Tigray zone. The shrub is habitat of altitudes ranging from 2500 to 3000 masl and grows on hilly and rocky land rich in black organic soil. In north Shewa, the survey was carried out once in a month for the whole year. Thus, aecial infections of the plants were not observed from February to June. In July, August and December, traces of aecia were noted, but from September to November aecial infections were abundant. The aecial samples collected were inoculated on seedlings of susceptible wheat, barley, rye and oat crops. After two weeks of incubation of aeciospores, stem rust (*P. graminis*) infections were recorded on wheat, barley, rye and rarely on oat crops. *P. graminis* that infected wheat, barley and rye have formae speciales *tritici/secalis*, while that of oat was formae speciale *avenae*. Moreover, some of the stem rust pustules found on these seedlings were inoculated on the sets of 20 wheat stem rust differential hosts and resulted in races avirulent to all/majority of the Sr. genes. The study indicated that *B. holstii* is an alternate host of stem rust. This report is the first of its kind in Ethiopia and further studies are recommended to determine the role of *B. holstii* in the epidemiology of wheat rusts in Ethiopia.

Keywords: Alternate host, Aecia, Barberry, *Berberis holstii*, Stem rust, *Puccinia graminis*

Introduction

Berberis is a genus of about 450-500 species of deciduous and evergreen shrubs from 1–5 m tall with thorny shoots, found throughout the temperate and subtropical regions of the world (apart from Australia). Species diversity is greatest in South America and Asia; Europe has a few species and North America two species. *Berberis vulgaris* is the most well-known species, which is common in Europe, North Africa, the Middle East, and central Asia.

Berberis vulgaris (European barberry) and *Berberis canadensis* (American barberry) serve as alternate host species of the wheat rust fungus *(Puccinia graminis)*, a grass-infecting rust fungus that is a serious fungal disease of wheat and related grains. For this reason, cultivation of *B. vulgaris* is prohibited in many areas and imports to the United States are forbidden. Many species of *Berberis*, *Mahonia* and their hybrid (x Mahoberberis) are susceptible to *Puccinia graminis*. Little specialization occurs on the alternate hosts, but is known to occur (Waterhouse 1929b; Green and Johnson 1958). Even on susceptible bushes only 2 weeks old leaves or less are normally infected (Melnder and Craigie 1927).

Two species of *Berberis* are present in Africa, *B. vulgaris*, naturally present in north–west Africa, but with a wider natural distribution in central and southern Europe and central Asia and *B. holstii* which, is endemic to the mountains of eastern and southern Africa. *B. holstii* is distributed in seven countries such as Ethiopia, Somalia, Kenya, Uganda, Tanzania, Zambia and Malawi (Whittimore 1997). The distribution coincides with the great chain of mountains and upland areas which run from the Ethiopian highlands to South Africa; these areas are of considerable phyto-geographical and ecological interest (Chapman and White 1970).

Flora surveys made by Addis Ababa University noted *B. holstii* in a book of the Ethiopian and Eritrean flora (Edward *et al.* 2000) and identified seven barberry sites in north Shewa, two in Wello and one in Tigray zones. The herbarium is preserved in Arat Kilo science campus, Addis Ababa University. Ample dried aecial infections were observed on the leaves of the herbarium. In 1978, researchers from Ambo Plant Protection Research Centre made the first attempt by inoculating aeciospores on 28 lines of small cereal crops, but there was no stem rust infection observed on these crops (SPL 1978). Therefore, this research was aimed at determining the possibility of stem rust of wheat infecting *B. holstii* as alternate host in Ethiopia.

Materials and Methods

Survey of Barberry Shrubs and aecia collection

Secondary data and the herbarium of Addis Ababa University were used to locate Barberry plants. In addition, the survey team used shoot samples of Barberry plants as a display to farmers to locate the bush in the vicinity. When the bush was located, all the necessary data such as region, zone, district, village, GPS coordinates (latitude and longitude), altitude, soil type and the presence of wheat and other cereal crops in the proximity were noted.

The plant parts (leaves, berries and stems) were examined for aecial infections. The

intensity of aecial infection was recorded each month as "no data" (if not surveyed), "no sign of aecia", "trace" and "abundant". Aecial samples were collected in paper bags and taken to the laboratory for pathogenicity analysis. Each sample in a bag was labelled. Additional information on name of the region, zone, district, village, GPS coordinates, accession number, date of collection, and name of the collector was recorded.

Preparation of seedlings for inoculation

To determine if aeciospores on leaves of *Berberis* spp. is pathogenic to wheat and other cereal crops ten susceptible wheat, rye, barely and oat genotypes were included in the study (Table 1).

To prepare one replication of this assay, two small pots (3.25" x 3.25" x 3.5" tall) were filled with soil: sand: compost in 2: 1: 1 v/v/v ratio. Five entries were planted into each pot and 5 seeds per entry (e.g. 5 seeds in each hole). Figure 1 shows the sowing layout for each pot and replication of the assay, including the position and labelling protocol for the pot stakes.

Table 1. Type of crops and varieties used to study the pathogenicity of aeciospores

Entry ID	Crop	Variety/genotype	Remark/source
1	Bread wheat	McNair 701	Stem rust susceptible
2	Bread wheat	Line E	Stripe rust susceptible
3	Bread wheat	Morocco	Stem rust susceptible
4	Bread wheat	Lemhi	Stem rust susceptible
5	Bread wheat	Sr31/6 *LMPG	Sr31 differential
6	Rye	Prolific	Stem rust susceptible
7	Rye	Winter rye	Stem rust susceptible
8	Barley	Hiproly	Stem rust susceptible
9	Barley	Hapana	Stem rust susceptible
10	Oat	Otana	Stem rust susceptible

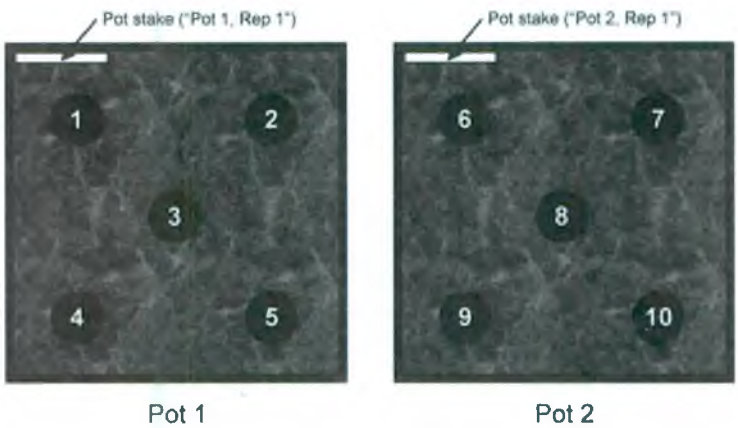

Figure 1. Sowing layout on each pot and replication of the experiment.

Once the seeds were sown, adequate soil moisture was maintained to promote germination and allow proper seedling growth. For watering, the pots were placed in a shallow tray of water rather than watering them from above. The pots were kept indoors at room temperature (~ 20 °C) during this time, under diurnal grow lights. The planted pots were placed in an area that was free from ambient cereal rust inoculum and was isolated from potential contamination by any rust. Besides, a negative control was included in parallel with this assay.

Inoculation of aeciospores

The seedlings were ready for inoculation 7-10 days after sowing; when the first leaf was fully expanded, but the second leaf not yet fully expanded. To inoculate one replication of the assay, the two pots were laid down inside a small tub such that the leaves intermingle, as shown in Figure 2.

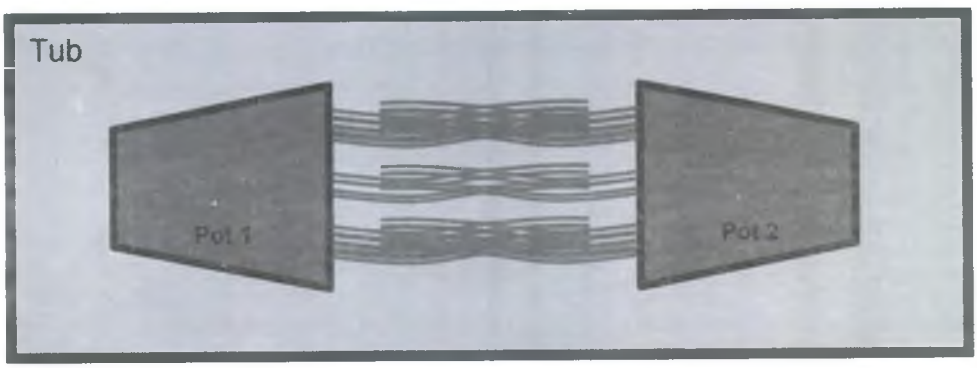

Figure 2. Layout of the pots in one tub of the assay.

The leaves were lightly misted with a dilute solution of Tween 20 (1 L water + 4 drops of Tween 20). A metal screen/grid was laid over the top of the tub and fresh aecia-bearing *Berberis* spp. leaves were laid on the screen, aecia-side down, as shown in Figure 3.

Once in place, the Barberry leaves were also lightly misted and then a damp paper towel was laid over them. At the point, the entire apparatus was placed inside a plastic bag/dew chamber and the bag/chamber was closed to maintain 100 % humidity. For stem rust the bagged tub was held at room temperature (~ 20 °C) in the dark for 24 hours. To accomplish this, inoculation was done in the evening and the bagged tubs placed outside/dew chamber.

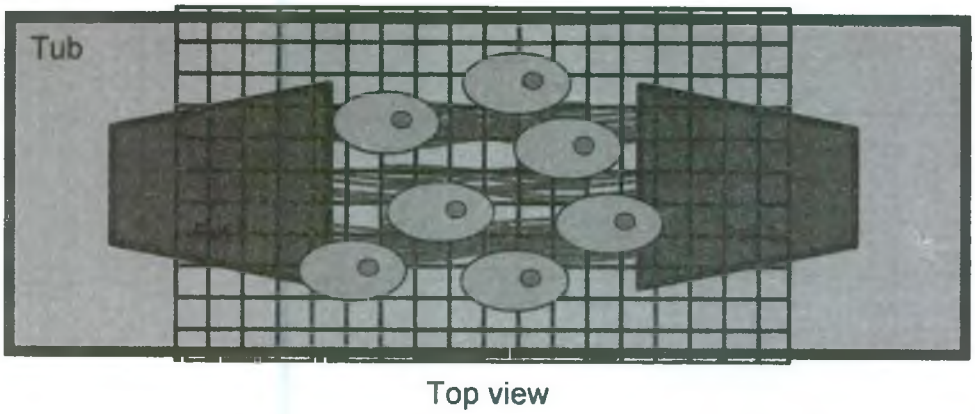

Figure 3. Inoculation process of aeciospores on the test seedlings in the tub.

After 24 hours, the chamber was opened, the screen removed, and the pots turned so that the seedlings that were originally lying on the bottom of the tub are now on top. The screen with the Barberry leaves were placed back on the tub, the damp paper towel put back on, and the whole apparatus placed back in the plastic bag/dew chamber for another 24 hours. Then, the pots were removed from the tub, placed upright, given a light misting, and the plastic bag placed over top of them, tent-like (open bottom), to facilitate a slow dry down, as shown in Figure 4.

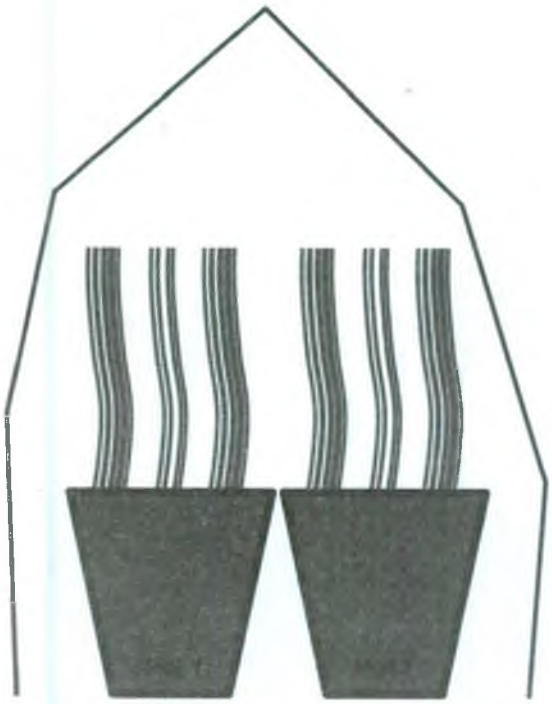

Figure 4. Aecia inoculated seedlings removed from the tub to slowly dry out.

To facilitate stem rust spore germination and infection the tented / chambered pots were placed in diurnal conditions near room temperature (~18-22 °C). After 3-4 hours, the bag was removed entirely and the plants were maintained normally (i.e. adequate soil moisture). Indirect sunlight is adequate for this stage. At 14 days of post inoculation, all seedlings were assessed for the presence/absence of stem rust pustules/infection.

Eleven stem rust pustules/isolates derived from inoculation of aecia on the identification series of wheat, rye, and barley crops were multiplied on the same crop species from where the pustules were taken. The multiplied isolates were then inoculated on the 20 standard stem rust differential hosts for race identification. After two weeks of incubation, the set was evaluated using 0-4 scoring scale (Stakman *et al.*, 1962) and the races were identified using the North American system of nomenclature of *P. graminis* (Roelfs and Martins 1988).

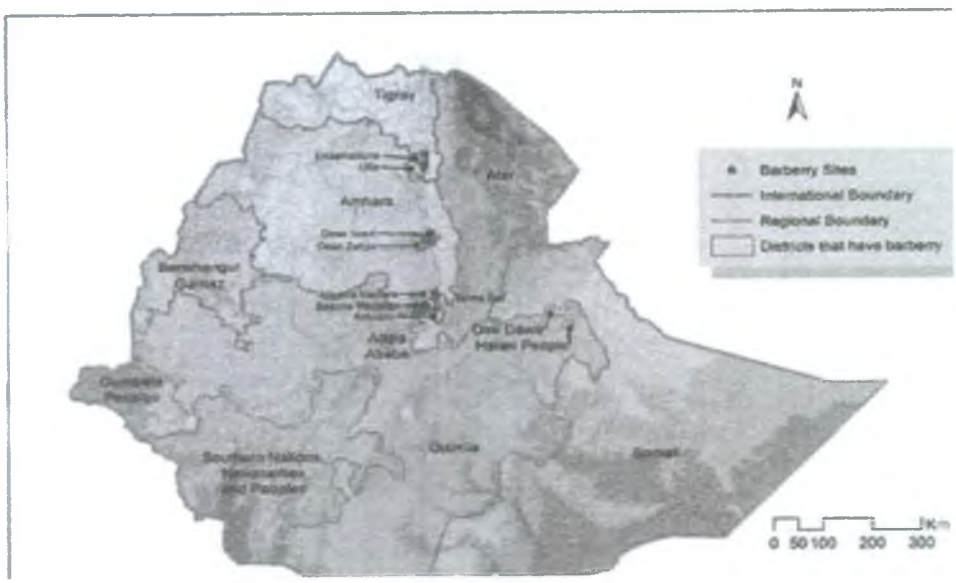

Fig.5. Map of Ethiopia showing the locations of Barberry shrubs.

Results and Discussion

Distribution of Barberry shrubs and aecial infection of the plants

Surveys were carried out a number of times in North Shewa zone since 2009 and a few times in Wello and South Tigray zones since 2012. The shrub is locally named as Zinkila in North Shewa and Yeset aff in Wello zones. Barberry plants were found in 19 localities, of which, 12 are in north Shewa zone, three in south Wello zone and four in south Tigray zone. The surveys encompassed nine districts, the majority being in north Shewa zone of Amhara region (Figure 5 and Table 2).

The shrubs grow at altitudes ranging between 2488 to 2979 m.a.s.l. The lowest altitude was in south Wello, Desse zuria zone and the peak was in north Shewa zone. The majority of the locations had altitudes above 2800 m, while a few had below 2600 m and these locations are in south Tigray zone. Latitudes for these locations ranged 09.61666° in north Shewa zone to 12.83693°N in south Tigray. The longitudes varied from 039.37092 to 039.75144°E (Table 2).

The locations where Barberry shrubs grow have light dark/black soil colour with black rocky stones. The plants grow in patches on hilly areas. Either wheat or barley or both crops as well as grass weeds grow around this alternate host. Some grasses were found infected with rusts.

The development of aecial infections on leaves/ other parts of Barberry plants was studied throughout the calendar year. In north Shewa zone, there was no sign of aecial infection on Barberry leaves/other parts of plants from the months of February to June. No survey was made in South Wello and South Tigray zones for the aforementioned period. In July, August and December, traces (not abundant) of aecia were observed whereas the aecial infection was abundant in September, October and November. Therefore, the later three months would be appropriate time for aeciospore survey in north Shewa zone. In south Wello zone, aecial infections were in abundance in November and December, while in south Tigary zone, the appropriate time for aecial infection development was not identified. In order to know the optimum time of aecia development on Barberry plants in south Wello and south Tigray zones, regular survey trips should be organized on monthly intervals.

Table 2. Survey of Barberry plants (*B.holstii*), the alternate host of wheat stem rust in Amhara and Tigray regions, Ethiopia, 2015.

Zone	District	Locality	Altitude (m)	Latitude	Longitude	Remarks
			Amhara region			
North Shewa	Ankober	Lay Gorebela	2829	09.65856	039.75144	35Km from D/Birhan
	Basona Worana	k.7/Birbirsa Gara Mite	2816	09.68532	039.46618	Enewari road to Goshe Bado, 4km
	Basona Worana	Mekegn Got,Genet Kebele	2815	09.68073	039.46061	
	Basona Worana	Abo Gedam	2702	09.68340	039.51389	
	Basona Worana	Atakilti	2818	09.67414	039.55880	
	Angolelana tera	Kule Bado, Faji	2896	09.61666	039.48289	120km from AAleft 3km
	Angolelana tera	Afaf, Faji	2886	09.61892	039.48554	120km from AAleft 3km
	Mendida	Adere Ejersa	2789	09.61680	039.37092	19km on Enewari road
	Mezezo	01 kebele	2860	09.94042	039.73335	
	Tarmaber	Womberme, Woyen bir kebele	2979	09.95898	039.72544	20km from Tarma ber
	Tarmaber	Yezeb Woyin	2987	09.88265	039.73177	
	Tarmaber	Sina Zuria	2764	09.86288	039.73177	
South Wello	Desse zuria	Abaso kota/k15/Gobeba	2488	011.07372	039.54386	To Gerado 12km
	Desse zuria	Yoguf, k 040 Hara Wobelo-kelina	2617	011.03669	039.64537	
	Desse zuria	Borusellase church	2756	11.2171	039.64760	10.5km from Desse
			Tigray region			
South Tigray	Ofla	Menkere	2742	12.57866	039.53796	1km east of Ashenge lake
	Ofla	Adi Bomosa	2559	12.61771	039.52672	
	Ofla	Ugam Burda	2538	12.63845	039.53881	Aba hawdela river
	Enda Mohoni	Ambahasti	2508	12.83693	039.55564	East of road to Mekele in adi emba village gerew chere forest

In these areas, cereals are sown in May and reach maturity growth stage after September. Grass weeds are in similar condition as cereals. At this time of the season, aecia production is in abundance and the possibility of infection of cereals and grass weeds by aeciospores is high. Sometimes stem rust infections were observed on wheat, barley and grass weeds grown in the neighborhood of Barberry plants, but they have not been analyzed due to the loss in viability. These rust samples should be collected and studied to compare with those races obtained from aeciospores.

Reaction of test crops inoculated with aeciospores from Barberry shrubs

After two weeks of incubation, small pustules of stem rust (*Puccinia* spp) were sporadically developed on the leaves and leaf sheaths of the test materials like Line E (wheat), Prolific, and winter rye (rye), Hiproly, and Hypana (barley) and Otana (oat). On wheat, barley and rye infections by stem rust were frequently recorded. Likewise, Line E a cross between wheat and rye is susceptible to wheat Pgt (*P. graminis* f.sp. *tritici*), and susceptible to most culture of Pgs (*P. graminis* f.sp. *secalis*). Very rare infection of stem rust, *P. graminis* with formae speciale *avenae* was noted on Otana (Oat).

Wheat *P. graminis* f.sp. *tritici* is closely related to rye *P. graminis* f.sp. *secalis* and they both readily hybridize with each other sexually and asexually. Similarly, the cereal and grass host ranges of *P. graminis* f.sp. *tritici* readily infects cereal rye (*Secalis cereal* L.) and many produce significant disease levels on some genotypes. Likewise, *P. graminis* f.sp. *secalis* infects and may produce high levels of disease on certain wheat genotypes (McIntosh *et al.* 1995). In addition, both formae speciales and their hybrids may be significant pathogen of barley (*Hordeum vulgare* L.) and various species of *Agelops* and *Agropyron* (McIntosh *et al.* 1995). However, since rye is not grown in Ethiopia and the grass weeds that are infected by both formae speciales (*tritici* and *secalis*) are similar. the stem rust that infected rye in our experiment could most probably be f.sp. *tritici (Pgt)*. On the other hand, these forms rarely, if at all hybridize with *P. graminis* f.sp. *avenae*. That is, the rare pustules that infected the susceptible oat variety could probably be oat stem rust. However, *P greaminis* f.sp. *avenae* was not detected on any of these hosts (wheat, barley and rye) under field conditions (McIntosh *et al.* 1995). The aeciospores infection of the test wheat, rye and barley varieties in the laboratory confirm that *B. holstii* surves as alternate host of stem rust pathogen.

Preliminary studies on eleven aeciospores derived stem rust isolates identified three races: BBBBB, GBBBB and DBBBB. The former was dominant (82 %) and avirulent on all of the 20 standard stem rust differential lines while the latter two races were virulent only on *Sr21* and *Sr9e* genes, respectively. For the first time, the preliminary results indicated that *B. holstii* detected as an alternate host to stem rust of wheat in Ethiopia. However, detailed studies should be carried out to investigate its role in the epidemiology of wheat rust in Ethiopia.

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