

The Causal Pathogen, Inoculum Sources and Alternative Hosts Studies of the Newly Emerged Gall Forming Faba Bean (*Vicia faba*) Disease in Ethiopia

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Abstract

Faba bean is the most important and dominant pulse crop of Ethiopia. In recent years the newly emerged disease were occurred and being threat to the production. The causal agent, disease alternative host and inoculum source were studied during the 2015-17 cropping season. As a result, based on field/plant symptom, morphometry and light microscopy, the pathogen were similar to previously identified faba bean gall disease which was caused by the fungal pathogen *Olpidium viciae*. It was observed that it can infect other hosts with mild severity. The alternate host study showed, the pathogen was largely soil and stubble born. Seeds from infected plant didn't develop any disease symptom the consecutive three years.

Keywords: Faba bean; *Olpidium viciae*; Microscope

Introduction

Faba bean is believed to be originated in the Near East and is one of the earliest domesticated legumes after chickpea and pea. Ethiopia is considered as the secondary center of diversity and also one of the nine major agro-geographical production regions of faba bean. According to Central Statistics Agency of Ethiopia 2013/14, Faba bean takes over 30% (nearly half a million hectares) of cultivated land with an average national productivity of 1.5 tons ha⁻¹. It is the first among pulse crops cultivated in Ethiopia and leading protein source for Ethiopian's and used to make various traditional dishes. Feeding value of faba bean is high and this legume has been considered as a meat extender or substitute due to its high protein content of 20-41%. From the economic standpoint, faba bean is a source of cash to the farmers and foreign currency to the country. Ethiopian farmers are also cognizant of the role of legumes in general and faba bean in reticular in improving soil health by fixing atmospheric nitrogen, and widely use them in rotation with cereals [1]. It occupies close to 574,060 ha of land with annual production about 943,964.2 tone [2]. Even though Ethiopia is the world's second largest producer of faba bean next to China, its share is only 6.96% of world production and 40.5% within Africa [3]. In Ethiopia, the average yield of faba bean under small-holder farmers is not more than 1.6 t ha⁻¹ [2], despite the availability of high yielding varieties (>2 t/ha) [4]. The low productivity of the crop is attributed to susceptibility to biotic and a biotic stress [1,5]. Of the biotic category, diseases are important factors limiting the production of food-legume crops as a whole and faba bean specifically in Ethiopia [6,7]. Diseases such as chocolate spot (*Botrytis fabae* Sard.), rust [*Uromyces Vicia fabae*(pers.) de Bery], Ascochyta Blight (*Ascochyta fabae* Speg.), black root rot (*Fusarium solani* Mart), and foot rot (*Fusarium avenaceum*) are among fungal groups that contributes to the low productivity of the crop [6,7]. Furthermore, a new emerging

disease which cause up to complete crop failure over wide areas within short period of time and aggravates the diminution of yield to maximum nationwide [5].

The disease is shortly covering areas of previously not observed. Yet, the disease is not well studied and its causal pathogen, spread mechanisms, and alternative hosts are not well identified. Thus, the aim of this study was to identify faba bean gall disease causal pathogen, inoculum sources and alternative hosts.

Materials and Methods

Study area

The experiment was carried out at North shoa, Addis Ababa University, Salale campus where the disease was first reported Dereje et al. [8] and the most faba bean gall disease hot spot area for three consecutive years.

Causal agent (pathogen) study

Faba bean gall infested fields around hot spot areas (highlands of North Shoa of Amhara and Oromia regions) and diseased individual plants were observed thoroughly to diagnose with its symptom. The formed gall were measured and recorded. Diseased leaves were crushed and set under light microscope for cellular examinations. All results were recorded and compared with previously documented characterization features of the pathogen.

Inoculum source study

Experimental setup: Micro plots with the volume of 1 m × 1 m × 20 cm were prepared on the wooden bench 1 m high above ground to avoid surface contamination. Each plot was filled with sterile soil. Infected faba bean residue (plant debris) from previously known field

and seed from different sources disease free seed and seed from previously infested faba bean field) were used to check for their potential inoculum sources. Ten plants were planted per plot and recommended fertilizer rate and weeding practices were done on time. The raised plots were partitioned by polyethylene sheet to protect the contamination from side plot by rain splash or wind current. Disease free seeds from Holetta research center were used (the place where the disease not observed). A total of four treatments were used in completely randomized block design and replicated three times. The seeds were planted following farmers planting time in the main season. Finally, faba bean gall disease incidence and severity were recorded.

Alternative host study

Experimental setup: Two meter long double experimental lines with 30 cm of intra line were prepared on precursor of diseased faba bean field with all its agronomic practices. The planting time follow farmers' season for faba bean.

Lotus corniculatus

Dolichos lablab

Vicia sativa (Vetch)

Trifolium quartianum

Medicago sativa var 5929

Vicia villosa 2441

Vicia dasycarpa var *lana* (vetch)

Medicago scutellata

Field pea (*Pisum sativum*)

Faba bean (*Vicia fabae*)

Fenugreek (*Trigonella sp.*)

Rape seed (*Brassica sp.*)

Lentil (sp.)

Chickpea

Seeds of all pulse crops were collected from disease free area (Holetta research center). Data was collected based on the presence and absence of faba bean gall disease symptom and under light microscopy.

Results and Discussion

Causal agent (pathogen) study

Identification of the causal agent study was largely depending on symptom descriptions and microscopic examination.

Field and plant symptoms of the disease

The infested fields were observed easily detected depending on their severity and stage of the plant. The disease observed soon after crop emergence and reaches its maximum in mid rainy season and gradually reduces as the season gone. In this moment the plants rejuvenate with new leaves and highly infected leaves were senescence. Severely infested field show highly stunted crops and the field turned dark brown (Figure 1C). Seriously infected plants are often stunted with few pods.

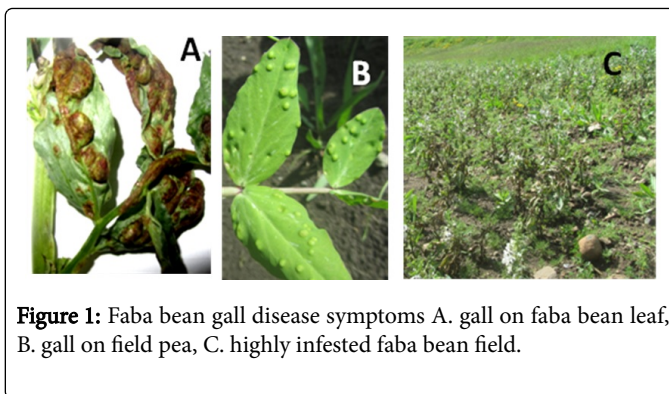


Figure 1: Faba bean gall disease symptoms A. gall on faba bean leaf, B. gall on field pea, C. highly infested faba bean field.

Faba bean gall disease symptom mainly observed on the leaf and stalk, sometimes also on petiole but not on pod. Its early appearance detected on the back (or sometimes front) side of leaves was light green nearly round bulge spots, the surfaces of the spots appeared rough with development, proliferated gradually to form chlorotic galls finally (Figure 1). The small tumors like galls are formed, 2-8 mm in diameter and 1-4 mm high before coalesced (Figure 1) which is comparable with Xing [9]. The individual gall was rolling up able to fuse into irregular shapes (abnormal growth of leaves) caused brown stain and necrosis of infected cells. Complete contraction and dysmorphism of the leaves were observed in its severe condition. At the later stage, the galls turn black or brown, the tissues decay and a few galls break to form necrotic areas. Leaves with more galls usually die earlier. These are considered as characteristic symptoms for the broad bean blister, which are the important basis for the diagnosis [10-13]. According to Dereje et al. the disease was first reported from North Shoa of Oromia region in 2010 and depending on its field symptom they suggest as it is "faba bean gall" incited by the pathogen (*Oplidium viciae* Kusano) Xing which was first reported as new species in 1912 in Japan [11,12] (Table 1).

Observations	Gall diameter (mm)	Gall depth (mm)
1	3	2
2	3	2
3	4	3
4	5	3
5	2	2
6	6	4
7	8	3
8	7	2
9	8	3
10	5	1
x	5.1	2.5

Table 1: The diameter and depth of galls on leaves in (mm).

The outbreak and distribution of faba bean gall were limited in ecological zone above 1800 m and above in Ethiopia the altitude suitable for faba bean growth.

Yan and Hua reported that, zoosporangiums were constantly reproduced after the disease occurred in field, liberated zoospores with the presence of rain or dew for secondary infection which cause highly repeated secondary infection [13]. The disease spread quickly in the field and reached peak outbreak around flowering and pod formation stages and then stopped gradually after pod stage.

Light microscopy of the disease

The pathogen infected and parasitized in the epidermal cells of leaf and stem. The host responded quickly to the invasion and then it induces symptom. The anatomy of epidermal tissues and cross sections of leaf and stem of broad bean, and the growth of the fungus in host cells were observed with light microscope. The pathogen of faba bean gall inhibited the epidermal cells of faba bean leaf, petioles and stalk. The fungus was unicellular and has one posterior tail (flagellum), with round protoplast. It was observed that the zoospores were able to function as motile isogamete and fused together or conjugates to form zygote with two cilia.

According to Yan and Hua the biological studies on *O. viciae* indicated, the mature zoosporangium in epidermal cells of disease spots was able to liberate zoospores once in the presence of rain water with germination temperature range of zoosporangium was from 0 to 18°C, it will be significantly inhibited below 18°C, and was unable to germinate at 20°C, 25°C and 30°C [13]. It was indicated as the zoospores were able to swim for 72 h between 0 and 5°C; for 24, 10 and 3 h below 10, 20 and 25°C, respectively; for only 5 min when temperature raised to 30°C [13]. The disease depended largely upon the temperature, gradually shortened with temperature increase with the optimum temperature for infection and morbidity was between 10 and 25°C.

From this diagnosis the pathogen is typical to the previously suggest pathogen by Dr Dereje and his team Dereje et al. [11]. The field symptom and laboratory examination also confirms the causal agent with that of *O. viciae* which was described from Japan and China [12,13].

Inoculum source study

The faba bean gall disease incidence and severity were recorded for eleven consecutive weeks from the date of seedling emergence. The disease symptom was observed on the second weeks after germination on disease infested soil and residues plots. The progress of the disease incidence gradually became sever as the rain increase (Figure 2). The incidence of each diseased plot was gradually increased and almost 81% for infested soil and 87% for crop residue at around the end of rainy season.

Healthy seed planted on faba bean crop residue and soil from the previously faba bean gall disease infested fields shown symptom starting from the first month and more sever in the middle of rainy season (in August). Eventually, as the rainy season end, the disease severity becomes less and the plant growth continues with relatively healthy looking shoots.

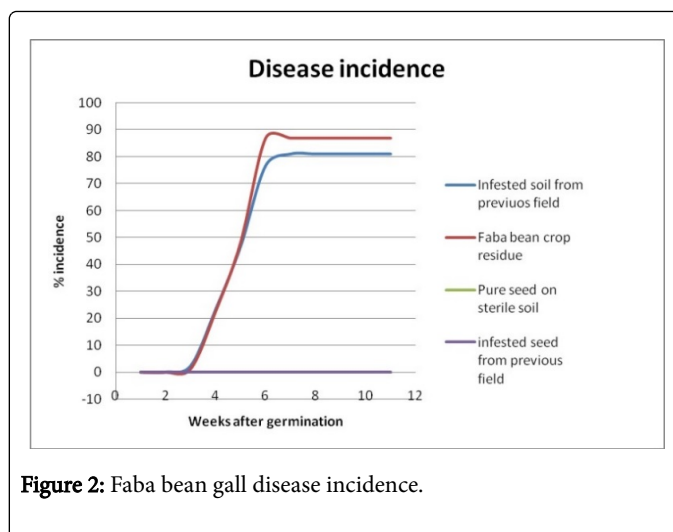


Figure 2: Faba bean gall disease incidence.

In this phenomenon, the total healthy leaf area was higher while disease progress curve goes down from the pick point (Figure 3). Seed from the same field (the field on which the test soil samples and residue were collected) didn't show faba bean gall symptom until the end of rainy season or crop maturity and also similar with that of control (pure seed planted on sterile soil).

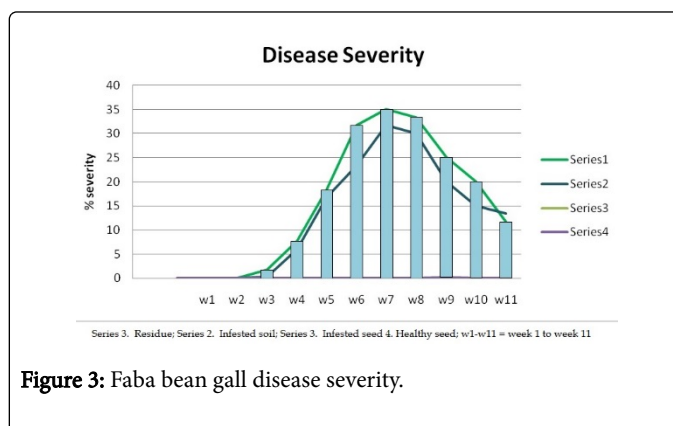


Figure 3: Faba bean gall disease severity.

This disease was reported to be able to spread in short distance by wind and rain. Continuous cropping and application of animal manure in which sick plant residue existed could cause this disease seriously [13].

Alternative host study

The faba bean gall caused by *Olpidium viciae* is known to infect some other crops in addition to faba bean. In Japan S. Kusano confirmed that the small galls cause the same disease which had a wide host range, including faba bean and pea. By artificial inoculation, Xing found that the pathogen can also infect rapeseed, cabbage, cucumber, spinach and buckwheat but not soybean, kidney bean and other legume crops [9]. On the faba bean disease survey, Hailu et al. found that field pea with gall symptom in Ethiopia [12]. The current study includes the most commonly cultivated highland pulses in Ethiopia. As a result the prior information on rape seed didn't observed in the growing season over all the studied period (three years). Field pea, lentil, vetch (forage crop) and grass pea shown gall symptom at lower severity (Table 2). Other tested pulse crops didn't develop observed gall

disease symptom. Field pea was the next highly infested pulse crop next to the control plot plant of faba bean and this crop is the most potential alternative host which the rotation avoid this crop. Or the field pea residue and field can be significant source of inoculums for the subsequent susceptible crop. Lentil and grass pea, even though the gall is observed at mild, their planting time in Ethiopia is late in the rainy season. While the late rainy season is unfavorable for this disease development, most likely, these crops may not be potential source for faba bean gall disease. Also, the disease may not a threat for these crops. Vetch the forage crop can be the potential inoculums source or alternative on which the disease can survive longer until it obtain its major host. The other study in china, faba bean gall disease resistance variety or cultivar didn't find [13]. Due to high soil moisture, three of the tested pulse forage plants and chickpea were not established.

S.NO	Legumes	Incidence	Severity %
1	Dolichos lablab	-	-
2	<i>Vicia sativa</i> (Vetch)	+	2
3	<i>Trifolium quartinianum</i>	-	-
4	<i>Medicago sativa</i> var 5929	-	-
5	Field pea	+	10
6	Faba bean	+	40
7	Fenugreek	-	-
8	Rape seed	-	-
9	Lentil	+	2
10	Grass pea	+	1

(+) Presence and (-) absence

Table 2: Tested pulse crops against faba bean gall disease.

Conclusion and Recommendation

According to the morphology, the organism was identified as *O. viciae* which is the disease agent of faba bean gall. It belongs to the genus *Olpidium* of the family *Olpidiaceae*, *Chytridiales*, *Mastigomycotina* in taxonomy.

Generally, from the result indicated, it is believed that the faba bean gall disease pathogen more survives in soil and crop debris/residues. Crop debris of the infested faba bean gall and soil of the previously year were found the most inoculums source of faba bean gall pathogen to transmit the disease to the subsequent season crop. As the control plot or disease free seed which was only on sterile soil and the seed collected from infested field didn't show any disease symptom, so far we have no information on its seed transmission and from our experiment the disease is believed to be neither air nor seed transmitted. But high current wind can transport with soil and crop debris particles [9,13]. Symptomless latent infection should be tested further.

From this experiment, faba bean gall disease cause a disease on faba bean as major host but still there are other pulse crops can be infected by this pathogen. Commonly cultivated highland pulse, field pea, is the most important alternative host. Field pea is mostly grown at similar time of planting season with faba bean and mostly around side field. More over all the farm machinery, farm animals and crop residue have higher chance of contamination with faba bean field. So, survival rate of the pathogen on crop debris and in the soil should be studied for proper crop rotation. Crop debris of these the diseased pulse crops should avoided and rotation should be done with cereal or other vegetable (potato) crops given enough time based on the study results.

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