SHORT COMMUNICATION

Grasspea Severe Stunt Virus Disease in Ethiopia

Adane Abraham and Alemu Lencho
Ethiopian Agricultural Research Organization, Plant Protection Research Center
P.O. Box 253, Ambo, Ethiopia.

Abstract

Symptoms suggestive of virus infection, notably severe stunting and yellowing, were observed in grasspea (*Lathyrus sativus* L) crop at different locations in the country. As high as 25 % incidence was observed in some farmers' fields although in most cases, typical symptoms were observed at a lower incidence. The causal agent was not transmitted by mechanical inoculation but was readily transmitted by aphids *Acyrthosiphon pisum* and *Aphis craccivora* from infected grasspea to healthy grasspea, faba bean, lentil, field pea and cowpea under greenhouse conditions. When field collected samples with severe stunting and yellowing symptoms were tested serologically against antisera of twelve viruses known to infect legumes by tissue blot immunoassay, most were positive for faba bean necrotic yellows nanovirus (FBNYV). Electron microscopic studies on selected serologically positive samples confirmed the presence isometric particles of about 20 nm diameter. No other virus was detected in the samples tested. This is the first report of a virus naturally infecting cultivated grasspea in Ethiopia and elsewhere. Due to heavy colonization of most grasspea fields with the pea aphid (*Acyrthosiphon pisum*), it is suspected that this aphid is most likely the natural vector of FBNYV in Ethiopia.

Introduction

Grasspea (*Lathyrus sativus* L.), locally known as "guaya", is one of the important food legume grown mainly in central and northern Ethiopia on about 142,170 hectares in 1997/98 (CSA 1998). In recent years, its cultivation is increasing because the crop tolerates stress conditions such as drought more than the other crops. The crop is cultivated in many tropical countries. It is generally known to have very few disease problems and no virus disease is reported to affect it (Brunt et al 1996). Recently however, a virus-like disease with severe stunting and yellowing symptoms was first observed in research stations and later in farmers' fields. This paper reports the symptom description and identity of the causal virus, its transmission by aphids to grasspea and other legume crops and preliminary information on its occurrence in farmers' fields in different parts of Ethiopia.

Materials and Method

Field Observation and Sample Collection

Severe stunting and yellowing symptoms in grasspea were first observed at Ambo Plant Protection Research Center field sites and nearby farmers' fields in 1996. Since then, some grasspea fields in different parts of the country were inspected for similar or other virus-induced symptoms. Samples were collected and either

blotted on nitrocellulose membrane or dried with $CaCl_2$ and brought to the laboratory for serological assay.

Serological Test and Electron Microscopy

To identify the causal virus, the collected samples were tested serologically by tissue blot immunoassay (TBIA) (Lin, et al. 1990) with some modifications. Antisera against twelve viruses known to infect legume crops were kindly provided by Dr. K.M. Makkouk, ICARDA, Syria. These were polyclonal antibodies to bean yellow mosaic potyvirus, broad bean stain comovirus, broad bean true mosaic comovirus, beet western yellows luteovirus, pea seed-borne mosaic potyvirus, pea early browning tobravirus, pea enation mosaic enamovirus, cucumber mosaic cucumovirus, chickpea chlorotic dwarf geminivirus, bean leaf roll virus and monoclonal antibodies detecting faba bean necrotic yellows nanovirus and luteoviruses. For TBIA, nitrocellulose membrane was blotted with stem or petiole sections and washed with 0.01 M phosphate buffered saline containing tween-20 (PBST). The membrane was blocked by incubating with 2 μg/ml polyvinyl alcohol for 1 minute followed by washing with PBST. The membranes were then incubated in a separate solution in petridishes containing each of specific antibodies for one hour with slight shaking. After washing again with PBST, the membranes were incubated for one hour with goat antirabbit (for polyclonal) or goat antimouse (for monoclonal) immunoglobulin conjugated to alkaline phosphatase and incubated as above. After washing, the membrane was developed by immersing in a mixture of 0.15 mg/ml bromo-chloro-indolyl phosphate and 0.30 mg/ml nitro blue tetrazolium in 100 mM tris containing 5mM MgCl2. The development of purple blue precipitate on the blots indicating the presence of the virus in the sample is observed either visually or using binocular microscope. Selected FBNYV-positive samples by TBIA were examined by JEOL JEM-100SX electron microscope at the Danish Government Institute of Seed Pathology, Denmark to confirm the presence of virus particles using 0.5% aluminium molybdate as stain.

Transmission Tests

Earlier in the study, attempts were made to mechanically transmit the causal agent through the sap of symptomatic grasspea leaves to seedlings of grasspea, pea, faba bean, lentil, chickpea and cowpea, and indicator plants such as *Chenopodium amaranticolor*, *C. quinoa and Nicotiana benthamiana* in insect proof greenhouse. Fresh leaves of the infected plant were extracted in 0.05 M phosphate buffered saline and rubbed in to the seedlings and inspected later for symptom expression. For aphid transmission tests, pea aphids (*Acyrthosiphon pisum*) and black bean aphid (*Aphis craccivora*) both collected from Ambo area and maintained in greenhouse on pea and faba bean plants, respectively, were used. Attempts were made to transmit the virus from infected grasspea to seedlings of legume crops; grasspea, pea, faba bean, lentil, chickpea and cowpea. Adult aphids were given acquisition feeding for two days on infected grasspea plants collected from Ambo farmers field, and about 10 viruliferous aphids were transferred to individual test plants grown in pots in the greenhouse. After an inoculation access feeding of two days, the aphids were killed by spraying actellic. The plants were then maintained in the greenhouse to allow symptom development. Plants were inspected for symptoms and later tested for FBNYV by tissue blot immunoassay as described above.

Results and Discussion

Faba bean necrotic yellows nanovirus was detected in most of the samples with typical severe stunting symptoms collected from various locations in Ethiopia. No other virus was detected in any of the grasspea samples tested. However, some samples with yellowing symptoms did not react with any of the antisera used. Table 1 shows all the locations surveyed and FBNYV incidence in such locations.

The electron microscopy of selected samples, serologically positive to FBNYV, revealed the presence of isometric particles measuring about 20 nm at very low concentration (about 10 particles per field). This agrees with the reported particle shape and size of FBNYV (Katul et al. 1993) confirming the presence of the virus in the samples.

Field observations showed that grasspea plants infected early with FBNYV, can be easily spotted in the field by very stunted growth and yellowing especially at leaf margins. The leaflets are very

small and fold to look as if the plant is blooming. The stem and leaves of diseased plants are stiffer and thicker than normal. Similar symptoms were observed in greenhouse inoculated grasspea plants . Such a distinct symptom can aid in field diagnosis of the virus in future studies.

Grasspea plants infected early were observed to die prematurely and thus do not survive up to the podding stage. In later infections however, plants were less stunted but leaf symptoms were visible and podding was variably reduced depending on the time of infection.

The fact that no other viruses were detected may be either due to the small number of samples tested or to our sampling procedure which was biased towards plants with typical yellowing and stunting symptoms. Detailed systematic survey in the future with randomly collected samples would help in evaluating the occurrence and relative importance of grasspea viruses in Ethiopia.

Attempts were made to see whether the causal agent is transmitted mechanically to legumes and indicator plants but it failed. Later, serological tests identified the agent as FBNYV, which is not transmitted by sap. On the other hand, the virus is readily transmitted by using aphid vectors *Aphis craccivora* and *Acyrthsiphon pisum* after two days each for inoculation and acquisition access periods from grasspea to grasspea, faba bean, field pea, lentil and cowpea. This agrees with the results of Katul et al. (1993) who reported these two aphid as the most efficient vectors of FBNYV. The virus could not be transmitted to chickpea however as the aphids did not feed long enough on this plant species to inoculate the virus. Otherwise, chickpea was kown to be infected by FBNYV (Katul et al 1993)

FBNYV is a recently described virus reported from West Asia and North Africa which infects many leguminous crops including faba bean, field pea, chickpea, lentil, haricot bean and cowpea (Katul. et al.1993; Franz et al. 1995). This virus was reported earlier from faba bean in Ethiopia from a field at Ambo (Franz et al. 1996) and later found to be the most widely distributed virus of faba bean (Abrahm and Lencho,1998). This is the first report of this virus or any other virus from grasspea in Ethiopia. Morever, no virus was recorded worldwide from cultivated grasspea (Brunt et al. 1996) although Mouhanna et al (1994) detected FBNYV from wild annual *Lathyrus* species.

The severity and death of infected grasspea due to the virus suggests that when environmental conditions are favourable for the aphid vectors, the disease is a potential threat to the expanding grasspea production in the country. In addition, since grasspea is grown in Ethiopia during the winter season after faba bean which is also affected by FBNYV, the virus and/or its aphid vectors may survive in this crop making the disease cycle to continue to the next season. The pea aphid which is shown to transmit the virus in this study is the major pest of grasspea, pea and lentil (Kemal, 1999). It is therefore suspected that this aphid may act as field vector of the virus in grasspea and possibly in other crops. Therefore, detailed study should be done in the future on the distribution and importance of the virus and its aphid vectors in grasspea, the epidemiological role of grasspea in virus survival and spread to other hosts like faba bean and the variability of grasspea genotypes in their reaction to FBNYV.

Table 1. Location where FBNYV was detected serologically from grasspea samples with severe stunting symptoms. Field incidence was based on visual estimation.

Zone/Wereda	Location	Altitude (m)	Incidence (%)
East Shewa			
Dalota	Debre Gella	2200	< 1
West Shewa			
Ambo	Gosu	2250	2
	Senkele	2250	5
	Meja	2225	20
North Shewa			
Mendi	Moye Melyu	2650	1
Debre Tsige	Chagen	2700	<1
Muke Turi	Kecha	2600	1
Sendafa	Dawi Giorgis	2575	<1
Waja Jarso	Abu Lencho	2500	1
Arsi			
Tosa	Gudercha	2350	<1
South Welo			
Haik	Zebit	1910	25
East Gojam			
Bahir Dar	Sebatamit	1950	2
South Gonder			
Kemkem	Addis Zemen	1950	<1
Leebo	Bura	1850	5
Fogera	Woreta	1825	<1

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