Present Situation of MLO diseases in Sudan

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Abstract

The earliest record on the occurrence of yellows-type diseases in Sudan dates back to late 1950's when Nour (1958) described a number of phyllody and witches broom abnormalities in Khartoum Province. Detailed investigations of the aetiology, diagnosis and epidemiology of these diseases has started in 1984. Thus during the past 10 years more than 20 new diseases have been described and the most economically important of which have been thoroughly studied. This paper highlights major advances in this field of plant pathology in Sudan with emphasis on diagnosis, epidemiology, control and prospects of future research.

Introduction

Early records of phytopathogens and their economic damage to major crops in Sudan goes back to 1918. Realizing yield losses incurred by diseases, research work in this field has become more organized thereafter, whereby systematic surveys, identification of pathogens and selection programmes were established. Despite that, many fungal, bacterial and viral pathogens have been identified, fully diagnosed and even reasonably controlled as early as 1950's, it was only until 1984 that the first MLO diseases were reported (Jones *et al.*, 1984).

During late 1970's and early 1980's, diseases resembling those caused by MLOs have increasingly become of concern in many crop plants, orchard and forest trees as well as weed and wild plant species. The need to elucidate their nature and etiology thus emerged as an important issue in order to better plan for their control. To attain this objective a comprehensive research programme was established in 1984/1985 which has involved the participation of many Sudanese and French Scientists. This paper therefore summarizes results of experimental work in the field, glasshouse and laboratory obtained during the past ten years most of which has already appeared as published papers and technical reports.

Geographical background

Sudan is the largest country in Africa with an area of about 2.5 million square kilometers. It extends along the Nile Valley from the tropical forest zone in the extreme south, shrubs and grassland savanna in the center to desert in the North. These different climatic zones are not sharply delimited as well defined micro-environments lie within these zones. This diverse climate gave rise to diverse vegetations and animal dwellers and consequently the type of agricultural systems and distribution of economically important crops. Thus, in the North where irrigated agriculture is practiced, date palms, citrus, wheat and cool season food legumes (faba beans, chickpea, lentils and peas) are grown. Cotton sorghum, groundnuts, sesame, sugarcane, millet, sunflower and castor seed in the central region and Mango cassava, Yam tea and coffee are grown in the South. Vegetables are grown throughout the country. However, wide scale commercial production of certain vegetables is restricted to certain regions depending on soil type, climate and sometimes availability of irrigation water. Certain micro-environments permit growth of temperate crop plants and fruit trees as is the case of Marra Mountains in the west and Ematong Mountain in the South.

The present investigation was mainly conducted in the central region of the country, Gezira State, which lies between the Blue and White Niles with an area of more than one million hectares. Here, cotton, sorghum, groundnut, sugarcane, wheat and various vegetables are extensively grown in well organized irrigated plots. Intensification and diversification of cropping is the main feature in this area. New crops which are traditionally grown in the cooler North of the country are now being introduced in this area e.g. wheat and faba beans. Hazards of desertification necessitated planting of wide areas with exotic quick growing trees such as Eucalyptus. This new, man-made, ecosystem seems to have provided favorable conditions for the abundance of a wide variety of pests and diseases. This has apparently also affected many yellows-type diseases which are now noticed to become epidemic in many plant species.

Diseases and their symptoms

Well before the first report by ISHIIJE *et al.*, (1987) of Mycoplasma-Like Organism (MLO) association with some diseases, many phytopathologists were convinced to place symptoms such as phyllody and witches' broom in a separate group designated as yellows-type diseases. This was despite the fact that they resemble diseases ascribed to viruses in many respects such as insect transmission. Likewise, symptoms have bean the main criteria used by NOUR (1958) to report phyllody of faba bean, *Crotalaria* spp., *Vinca rosea* and sesame from Sudan. During the course of this investigation, a preliminary survey was conducted in early 1985 in which faba bean and *Crotalaria phyllody*, virescence of *vinca* and *Zinnea elegans*, little leaf of *Eucalyptus*, white leaf of Bermuda grass were described and diagnosed as MLO diseases. Following this, 16 other diseases (table 1) including tomato big bud, tomato witches' broom, phyllody of Ipomea and Matat, witches' broom of pigeon pea, groundnut... etc were reported (DAFALLA, 1991). More recently, four more diseases, Neem witches' broom, lime witches' broom sorghum yellow stunt and hot pepper sterility, were diagnosed as MLO diseases (DAFALLA, unpublished).

Table 1. List of MLO diseases reported in Sudan until 1993

Family	Species	Common name*	Symptoms	Method of diagnosis
Malvaceae	*Abutilon figrianum*	Hambuk	Witches' broom, virescences	Tr. FM
	Sida alba	Um Shidayda		FM
Leguminosae	*Rhinchasia memnonia*	Adana	Phyllody	FM
	Crotalaria pycnostachya	Sofira	Phyllody, witches' broom	FM, Tr.
	Vicia faba	Ful Masri	Phyllody, Yellowing	FM, Tr. (S-?).
	Cajanus Cajan	Lubia Adasi	Witches' broom, sterility	FM
Tilliaceae	*Sesamum indicum*	Simsim	Phyllody, witches'broom	FM, Tr
	Chorchorus olitorius	Khudra	Yellowing	Tr
Convulaceae	*Ipomen cardiosepala*	Hantut	Phyllody, Witches'broom	FM
	I. cordofana	Tabar	Phyllody	FM
Compositae	*Sonchus cornutus*	Moleita	Virescence, reddening	FM
Solanaceae	*Lycopersicon ulentum*	Tamatim	Bigbud	FM, TEM. Tr.
	L. esculentum	"	Phyllody witches'broom	FM FM
	L. esculentum	"		
	L. peruvianum	?	Big bud	Tr. FM
Convolulaceae	?	Matat	Phyllody	FM

* Common names in the Gezira area.
Tr. = graft transmission,
FM = Fluorescence microscopy,
TEM = Transmission electron microscopy,
S = Serology.

With regard to symptom expressions, certain symptom types appeared to prevail in a particular group of plants than another. Thus in herbaceous plants such as faba bean and *Ipomea* sp, predominant symptoms comprise various degrees of virescence, phyllody floral and foliar proliferations and reduced leaf size. When the plant receives infection after fruit formation it has been noticed that seeds tend to germinate inside immature fruits. In this group the entire plant including the root system is affected and become completely sterile. In trees such as Eucalyptus and Neem infection is usually partial and most prevalent symptoms comprise leaf yellowing, little leaves, stunting, witches' broom and sometimes necrosis and gumming of the trunk. Grasses such as sorghum and Bermuda grass present a group in which yellowing and stunting are the most dominant symptoms.

MLO diseases tend, in certain cases, to prolong vegetative cycle of infected plants. For instance, in western Sudan phyllody-infected sesame plants were abundantly seen in January-February in fields where the original crop has already been harvested during October-November. Likewise, infected faba bean plants were kept under glass house conditions for extended periods of more than seven months. Certain climatic and soil factors, on the other hand, tend to aggravate infection with MLOs resulting in premature death of the plant. Eucalyptus plants infected with little leaf (DAFALLA & COUSIN,1986) were noticed to be extremely sensitive to drought whereby high proportion of dead trees were recorded during summer months.

Diagnosis

Various biological techniques including symptom development, transmission and use of differential host plants were employed. Faba bean phyllody (FBP) MLO was experimentally transmitted by grafting to faba bean and French beans and typical symptoms were observed. Dodder transmission using *Cuscuca subinclosa* was made from FBP to *Catharanthus roseus* and the symptom development followed in the later resembled that of Ash yellows in Europe and Crotalaria from Sudan. Symptoms are, however, not always reliable for precise diagnosis of MLO diseases. Certain criteria were set forth and agreed upon by various scientists to ascribe MLOs to a particular disease. Of these, the presence of pleiomorphic MLO bodies in the phloem seive tubes of affected plants is the most important. This is usually done in thin sections observed by transmission electron microscopy. Although the use of this technique is essential to initially associate MLOs with a particular disease, it could not be used for routine diagnosis as it is laborious and time-consuming. Methods of fluorescence microscopy using DNA-specific dyes (DAPI) and thick sections prepared from diseased plants were successfully employed to detect MLOs. This technique has been much improved and widely used during these investigations. For instance, fresh prepared cryosections of 3-15 μm, section from paraffin or historesin-embedded preparation were successfully used to diagnose many MLO diseases from Sudan (DAFALLA and COUSIN, 1986, 1988a, 1988b, 1988c, DAFALLA and FREINGOUN 1991,1992). This technique is extremely sensitive in herbaceous plants but has some limitations in ligneous plants due to small size of sieve tubes and low concentration of MLOs (DAFALLA, 1987). This technique, though sensitive and less laborious has the obvious disadvantage of being non-specific. COUSIN *et al.*, (1986) developed immunofluorescence technique in which they compare primary or endogenous fluorescence, secondary fluorescence (DAPI) and FITC in sections incubated with specific antisera. The technique permitted differentiation of FBP MLO and stolbur MLO and proved more reliable than DAPI technique. Moreover, it has been successfully used to screen supernatants of hybridoma cells lines raised to produce monoclonal antibodies against FBP (GOSCLAUDE *et al.*, 1986). Significant improvement of serological detection of FBP MLO was brought about through the use of F (ab') 2 fragment and a polyclonal antiserum (SAEED *et al.*, 1992). In addition to ELISA, immuno-transfer techniques and subsequent characterization of certain MLO immunogenic proteins then followed (SAEED *et al.*, 1992). Serological techniques have, however, failed to defect MLOs in trees. The use of DNA probes and PCR was therefore thought of as the technique of choice in this case. Specific probes were prepared for MLOs of poplar witches' broom (COUSIN-unpublished). The technique was also explored for FBP MLO from Sudan whereby two probes, one of 1.2 Kbp and the other of 1.45 Kbp were successfully used (SAEED *et al.*, 1992). In addition, two primers were prepared from FBP MLO and used to perform PCR.

Epidemiology

Various aspects of the epidemiology of MLO diseases in Sudan have been the subject of many studies. Transmission and spread of such diseases are usually mediated by insects, namely leaf hoppers, members of the family cicadellideae. Only few members of this family have been described and classified in Sudan, and most of which are not known as MLO vectors.

Investigations have therefore started with attempts to identify existing species and subsequently to study their role in MLO transmission. Two species, namely *Eusclidius variegatus* and *neolimnus aegypteacus* were identified and used in a series of tests for the transmission of FBP MLO. Initial results indicated that *E. variegates* could acquire MLOs diseased plants but were not able to transmit them to healthy test plant (MOREAU and KHOGALI, 1992; KHOGALI & MOREAU, 1992). The presence of MLOs in the abdomen, but not salivary glands of the insect, was proved using ELISA and FBP MLO specific antiserum (KHOGALI & MOREAN, 1992).

Studies of genetic relatedness within MLOs causing various diseases in Sudan has been initiated using similarities in symptoms expressions in *Catharathus roseus* as a standard differential host plant. These studies showed that FBP MLO is identical to crotalaria phyllody MLO but different from vinca phyllody collected from the same site in Sudan (DAFALLA & COUSIN, 1988c). Using ELISA and FBP MLO-specific antiserum, SAEED *et al.*, (1992) concluded that FBP and crotalaria phyllody are caused by the same MLO. They further indicated that cortalaria phyllody MLO from Sudan is serologically unrelated to witches' broom MLO of *C. Juncea* from Thailand. However, using FBP specific probe positive reactions were obtained (SAEED *et al.*, 1992).

Field studies were mainly concentrated on FBP in the Gezira as it is the most important and most highly epidemic. The crop was recently introduced in the central region (Gezira and Rahad Schemes). Incidence levels in this area were noticed to exceed 25% in certain seasons, compared to less than 1% in traditional production areas in the North. Figures of about 8-15% were reported during the past six years in the Gezira. No signs of varietal resistance were noticed in more than 200 varieties and lines (DAFALLA & FREIGOUN, 1991). Studies of temporal and spatial patterns of spread of FBP under natural field conditions were studied by DAFALLA & FRIEGOUN (1991) and DAFALLA (unpublished). Due to the short winter growing period choice of sowing date is limited, however, it appeared to be an important factor in disease incidence and rate of progress. Thus, it was found that total incidence in faba bean sown in the first week of November is significantly less than early sown crop in mid-October. Likewise, the progress of infections is faster in the latter case. Spatial pattern of spread seems to follow the direction of North-East winds prevailing during winter. This was the case when infections were periodically followed from the beginning of the season or when the relative distribution of infections in the whole field was assessed at the end of the season (DAFFALA & FREIGOUN 1991).

Conclusion

Plenty of information on MLO diseases have accumulated during the past ten years. Despite this, many basic aspects are not yet fully understood, most important of which is natural spread of the disease through insect vectors. In a recent report of a survey organized by ICARDA, faba bean phyllody was noticed to be the major disease problem in non-traditional production areas (Gezira & Rahad) (DAFALLA *et al.*, 1994). Attempts should be made to get hold of its vector and consequently design control strategies against it. In citrus orchards many decline diseases have been attributed to MLOs, however, due to limitations in diagnosis in trees, these diseases are still of uncertain etiology.