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Characterisation of viruses of pepper (Capsicum spp.) and sweet potato (Ipomoea batatas) from Ethiopia

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Introduction

This part consists of two sections: The first section outlines general background information and objectives for conducting this study. In the second section, literatures about the investigated crop plant virus pathosystems are reviewed in detail.

Ethiopian economy is mainly based on agriculture. Pepper *(Capsicum* spp.) and sweet potato (*Ipomoea batatas*) are two important crops in Ethiopia. The steady decline in price of other Ethiopian main cash crops such as coffee, requires diversification of agricultural export commodities. In this regard, pepper and sweet potato could play important roles, if constraints are identified and productions are geared to more commercial oriented activities. Lessons could be learnt from other countries. For instance, around 20% of pepper and 88 % of sweet potato world production comes from Indonesia and China respectively (HIJMANS *et al.,* 2001; FAOSTAT, 2002; FAOSTAT, 2003)

Chilli pepper dominates the world's hot spice trade and is grown everywhere in the tropics as well as in many temperate regions on the globe. Sweet potatoes are vital staple and commercial crops. Because of enormous importance of both crops, the Asian Vegetable Research and Development Centre (AVRDC) in Taiwan and International Potato Centre (CIP) in Peru give highest priority to international research in pepper and sweet potato, respectively.

Hot pepper is produced all over Ethiopia but districts in the rift valley in the south are the major production segments. All domestic consumptions in the country come from production by subsistence farmers. Economically, it is also an export crop. Pepper has a unique traditional place, as dried powder from its pods is condiment of the daily diet of Ethiopian food. In addition, small-scale farmers use pepper to generate income for various on- and off-farm economic activities.

Sweet potato is produced mainly in the southern and eastern parts of Ethiopia. It is a strategic crop, safeguarding farmers when the food reserves from cereals fall back. Nationally and internationally, sweet potato continues to be an important crop as alternative to cereal grains, mainly because of its high starch content (CIP, 1999b). For example, in the last 10 years, the area of sweet potato cultivation has increased from 19000 ha to 25000 ha in Ethiopia (FAOSTAT, 2002). Since recently, sweet potatoes with yellow and orange-fleshed tubers are widely promoted in east Africa including Ethiopia in order to alleviate Vitamin A deficiency, due to their high beta-carotene content (CIP, 1999a; HAGENIMMA *et al*., 1999).

Frequent and severe outbreaks of viral diseases in hot pepper and absence of even one single study in the field of sweet potato virology in Ethiopia have prompted this study. *Capsicum* spp. constantly shows severe virus symptoms throughout Ethiopia and in the rift valley districts particularly. Farmers in general, state farms in the past and recently private investors have often banned the production of the crop because of unacceptably high losses due to viral infection. Thus, yield and production areas of pepper remained constantly low in Ethiopia over the last 10 years (FAOSTAT, 2002). Since some years the situation is reflected by inflationary increase in price of the crop. Production deficit caused the appearance of forged pepper powder products in local markets that were harmful to human health.

It is worthwhile to see, however, that studies on these viruses are scarce and the few studies (AGRANOVSKY, 1993; YAYNU, 1998) available, are limited to virus diagnosis and identification, and are even contradictory (ADANE and HABTU, 2000). Confirmation and follow-up studies that generate reliable background information are lacking. Investigations depicting importance of each virus or virus isolate would help to prioritise the problems. As virus isolates and species can vary seasonally and spatially, continued survey and monitoring investigations are essential. Such basic information is necessary and required as prerequisite to design location specific management measures against plant viral diseases (HULL, 2002).

Viruses are the major problems of sweet potato production worldwide. Apparently, because of lack of diagnostic facilities, as well as trained plant virologists, there are no studies on sweet potato viruses in Ethiopia and their status is rather unknown. For purposes of crop breeding and improvement, there is an intensive exchange of sweet potato germplasm. Most of the germplasm used for crop improvement in Ethiopia has been introduced from other countries (ARC, 1999). Although international rules and regulations ban the exchange of infected material, viruses in sweet potato remain undetected and there is always a danger of their introduction. The problem could even be more fatal in countries such as Ethiopia that lack adequate quarantine systems. Viruses introduced with planting material could accumulate through time and cause crop health and yield deteriorations. Since sweet potatoes are reproduced vegetatively, exotic viruses infecting the crop can result in epidemics (MOYER, 1987). Such an increase of viruses in sweet potato planting material through time has been well documented (ONWUEME and CHARLES, 1994; KARYEIJA *et al*, 1998b). Hence, rapid detection of exotic- and identification of endemic- viruses is vital in the prevention of viral outbreaks.

Generally for any efficient control and breeding program, it is a prerequisite to identify and characterise viruses occurring in a particular geographic area and to obtain knowledge of their epidemiological characteristics. This study intended therefore to gain a better understanding of the dynamics of epidemics and the viruses involved in infection of pepper and sweet potatoes in Ethiopia. More specifically, the objectives were to:

- further identify and characterise viruses of hot pepper
- map the distribution and the prevalence of the viruses

- study variability of relatively important pepper viruses
- study roles of primary (alternate hosts, seeds) and secondary (vectors) sources of infection
- evaluate resistant lines against the most important virus infecting hot pepper
- survey and identify viruses infecting sweet potato
- characterise viruses infecting sweet potato biologically, serologically and molecularly.

1 The crop plant virus pathosystems

In this section, information about pepper, sweet potato and the most important plant viruses infecting both crops is reviwed.

1.1 *Capsicum* spp.

The genus *Capsicum* represents a diversified plant group from the well known sweet green bell pepper to the fiery hot, recently exploited habanero (BOSLAND, 1996). Pepper is a member of the family *Solanaceae* that includes tomato, potato, tobacco and petunia. *Capsicum* consists of about 22 wild species and five domesticated species (Bosland, 1994). It probably evolved from an ancestry in the Bolivia /Peru area (HEISER, 1976). In addition, wild peppers presumably occurred in Middle America, Amazonia, South America and the West Indies (ESHBAUGH *et al.,* 1983). Secondary centres of diversity exist in south and central Europe, Africa and Asia (IBPGR, 1983). Studies reveal the presence of locally adapted lines in India (MEHRA AND PETERS, 1991) Ethiopia (ENGELS, 1984), Turkey (ALAN, 1985), Czechoslovakia (PENETHEVA, 1987), Spain (NUEZ *et al.*, 1992), Italy /Mediterranean region (NUEZ *et al.,* 1992) and Nigeria (DEONTON AND MAKUNDE, 1993). The spread of Chilli in the world is historically associated with the voyage of Columbus (HEISER, 1976) to whom the credit for introducing pepper to Europe and subsequently to Africa and Asia is given (BOSLAND, 1996). Peppers have now spread throughout the American, African and Asian tropics. They are grown extensively under various environmental and climatic conditions in more than 60 countries (MARTELLI and QUACQUARELLI, 1983). Today five species of *Capsicum* are cultivated (IBPGR, 1983; PICKERSGILL, 1997). The *C. annuum* complex, which includes three closely related species, *C. frutescens* and *C. chinense* are the most widely grown in the Americas and worldwide. *C. annuum* that has been domesticated in highlands of Mexico, includes most of the Mexican chilli peppers, most of the hot peppers in Africa and Asia and the various cultivars of sweet peppers grown in temperate countries. *C. frutescens* and *C. chinense* are cultivated in Africa and Asia as spice crop, as intact fruits or for their oleoresin content. The other two species namely *C. baccatum* and *C. pubescens* are predominantly confined to Latin America (PICKERSGILL, 1997). Despite their vast trait differences, most Chilli cultivars commercially grown in the world, belong to the species *C. annuum* (BOSLAND, 1994). The total area devoted to pepper is estimated to be 4 million ha with an average annual increase of 5 percent (FAOSTAT, 2002). World pepper production reaches about 11 million tons of which 4.3 million come from Asian countries such as India, Indonesia, China and Korea (AVRDC, 1999; FAOSTAT, 2002). In many countries of the world, pepper is a cash crop with high domestic- and export value. Chilli industry has grown from regional food supply for tourists to an international industry, competing on the global market. At present there is an expanding pepper market for dietary consumption. *Capsicum* has more uses than any other vegetable (BOSLAND, 1994). Pepper is rich in vitamin A, B and C, potassium, phosphorus and calcium. It especially contains more vitamin C than any other vegetable crop (DEWITT and GERLACH, 1990). Paprika and oleoresin are currently used in a wide assortment of foods, drugs and cosmetics as well as for improving the feather colour of flamingos in zoos or koi fishes in aquariums (BOSLAND, 1994). The pharmaceutical industry uses capsaicin as a counter irritant balm for external application and to alleviate pain (CARMICHAEL, 1991). Because of their unique fruit shapes and bright fruit color, several pepper species have been widely used as ornamentals (BOSLAND *et al.,* 1994).

In Ethiopia the very common type of *Capsicum* is hot pepper or Chilli pepper. Portuguese had probably introduced hot pepper to the country in early 17th century (HUFFNAGEL, 1961). It has since been grown as important spice and vegetable everywhere. Today, the crop has not only attained economical, but also traditional importance. It is one component of the daily diet of Ethiopian people. Traditionally, Ethiopians distinguish three kinds of hot peppers; “berbere” the red mature pungent fruits, “karya” the immature green fruits and “mitmita” the small very pungent fruits. The powder from dried ripe fruits of hot pepper is used as spice to flavour ‘Wot’, an Ethiopian stew in a daily traditional meal. The fully mature green pods are eaten as salads. Meals without pepper are considered as tasteless to many Ethiopians. The crop is exported as dried ripe fruit or as oleoresin extracted from the fruits (JACKSON, 1987; YOSEF and YAYEHU, 1989). In addition, it serves as income- generating crop for small-scale farmers. Production of pepper is scattered all over the country. The pepper production area in the country is estimated to be about 340,000 ha (FAOSTAT, 2002). Today, small-scale farmers produce the largest proportion of hot pepper in the country. Districts in the southern and Oromyia regions, situated in the rift valley are some of the main production sites. In many areas, pepper is grown predominantly as monocrop, and rotated with cereals or legumes, using the main rainy season. However, pockets of production in the dry season using irrigation can be found, particularly in the rift valley parts of Ethiopia. Ecological isolations in hot pepper growing areas are common and there exists high variability in growing conditions and cropping systems. The crop is mostly grown in medium altitude (1400-

2000m) above sea level on sandy loam or clay loam soils with enough moisture capacity (YOSEF and YAYEHU, 1989). Due to adaptation to different agro-ecological zones, many local genotypes have evolved with fruits ranging from very long and broad types to very small and pungent types. Farmers use to grow mostly local selections, because there are shortages of improved varieties. Seeds are either from former harvests or bought from local markets. Seed lots are often mixtures of different varieties.

1.2 Sweet potato (*Ipomoea batatas*)

Sweet potato (*Ipomoea batatas* L. (Lam) is a dicotyledonous plant, which belongs to the family *Convolvulaceae* (AUSTIN, 1987). The family contains about 55 genera and more than 1000 species are described (WATSON and DALLWITZ, 2000). Of the family *Convolvulaceae*, *I. batatas* is economically the most important crop worldwide (ONWUEME and CHARLES, 1994). In addition, *I. aquatica* is grown as food plant in some Asian countries (WOOLFE, 1992). It is believed that *I. batatas* probably originates 500 years ago from a cross between *I. trifida* and another wild *Ipomoea* species in Central- and South Americas (JARRET and AUSTIN, 1994; HUANG and SUN, 2000). The crop may have arrived in Africa in the post Columbus era (YEN, 1982). Many authors suggest, that Spanish explorers took the crop to Spain early in the sixteenth century and later to Africa from Spain (YEN, 1982). The countries of Guatemala, Colombia, Ecuador, and Peru have the greatest diversity in sweet potato germplasm (AUSTIN, 1987). Secondary centres of genetic variability are Papua New Guinea, the Philippines and East Africa (YEN, 1982). Today, thousands of cultivars of sweet potato are grown throughout the tropics, subtropics and temperate regions (HE *et al.,* 1995; HIJMANS *et al.,* 2001). Sweet potato has been gaining a tremendous importance all over the world. According to FAOSTAT (2003) the total cultivated area of sweet potato is more than 9 million hectars. Over one hundred developing countries cultivate sweet potato and it is the most important food crop in over 50 countries (FAOSTAT, 2003). In developing countries, sweet potato ranks as the fourth most important food crop after rice, wheat and cassava on fresh-weight basis (FAOSTAT, 2001). More than 95% of global sweet potato production comes from developing countries (FAOSTAT, 2001). China supplies about 88% of world's production, making the country the leading producer in the world (HIJMANS *et al*., 2001; FAOSTAT, 2003).

In Africa about three-quarters of sweet potato production is located in East Africa, particularly around the great lakes region (KARYEIJA *et al*., 1998b; HIJMANS *et al.,* 2001). It is indicated that Rwanda has the greatest per capita consumption and Uganda has the greatest production in Africa and the second largest in the world (FAOSTAT, 2003). Sweet potato is adapted to a broad range of agro-ecological- and production conditions. Small- holder farmers grow sweet potato widely in sub-Saharan Africa (HORTON, 1988; CAREY *et al*., 1999). The crop is increasingly becoming an important famine relief crop during crop failure (CIP, 1998). In subsistence farming systems sweet potato has been very attractive and extremely important, because of its adaptation to low input agricultural systems, tolerance to drought, high yield per unit area, ease of establishment from vine cuttings and production with little input in terms of fertilisers and pesticides (WOOLFE, 1992). In addition, sweet potato has a great potential to suffice specific uses because of its enormous genetic diversity as well as morphological and phenotypic traits (ZHANG *et al.*, 1998; 2000; WOOLFE, 1992). Sweet potato gives a high biomass production per unit of land and time (WOOLFE, 1992). The roots provide energy, protein, vitamin B1 and B2, niacin, pyridoxine, folic acid, ascorbic acid, nutrients, including calcium, phosphorus, iron, sodium and potassium (WOOLFE, 1992). The crop produces larger amounts of edible energy than other starch staple crops such as rice, maize, cassava, banana, sorghum, yam and millet (WOOLFE, 1992;). Young sweet potato leaves are a rich source of vitamin A, B_2 and proteins (WOOLFE, 1992). Additionally, sweet potatoes are widely used as livestock feed (CIP, 1998)

It is not documented at which time the cultivated sweet potato was introduced to Ethiopia and cultivation has started. Since the genus *Ipomoea* is naturally pantropical (KARYEIJA *et al*., 1998c) many wild species are also identified to exist in the country (FERDU, 1999). In Ethiopia, sweet potatoes are mainly produced in the southern and eastern parts of the country. It is shown that the area covered by the crop can reach 49 000 ha with a total annual production of 200,000 metric tons (FAOSTAT, 2000). 63% of the total production of sweet potato in Ethiopia comes from the southern region (OA, SNNPR, 1999). In this region, sweet potato is the second most important root crop next to enset (*Ensete ventricosum*) in area coverage and to maize in consumption (TEREFE, 1995; ARC, 1999). Major zones of sweet potato production in the southern region are Welayita, Kembata/Tembaro, Gamogofa, Sidama, Gedeo, Dawuro and Hadiya. Most of these areas are known for a high population density. In these areas, sweet potato serves as security crop, when the food reserves from cereals dwindle. The period between September and November, the end of the rainy period, is the main planting time for sweet potato, although successive planting is practiced as well. Like in most sub-Saharan countries, sweet potato production systems in Ethiopia are not commercially oriented, but sweet potato is primarily produced as food insuring crop to low-income farmers.

1.3 Overview of Potyviruses in general

Potyviruses (genus *Potyvirus*; family *Potyviridae)* are the largest group of viruses infecting plants and include nearly 200 definitive and tentative members (VAN REGENMORTEL *et al.,* 2000). Potyviruses occur worldwide, but are most prevalent in tropical and subtropical regions. Most of them infect dicots and a few infect monocots. They are the most destructive viruses of cash crops (SHUKLA *et al.*, 1994). Potyvirus virions are non-enveloped, filamentous particles, 680 to 900 nm long and 11 to 15 nm wide (DOUGHERTY and CARRINGTON, 1988; LANGENBERG and ZHANG, 1997). Potyviruses are quite similar in terms of their genomic structure and strategy with bipartite Como- and Nepoviruses and the animal Picornaviruses (GOLDBACH, 1986). The genome of potyviruses has a single stranded positive sense RNA of approximately 10 kb, with a 5' terminal linked protein (VPg) (HARI, 1981) and a 3'poly A tail (MURPHY *et al.,* 1990). The RNA genome contains one long open reading frame (ORF) expressed as a 350 KDa polyprotein precursor (RIECHMANN *et al.,* 1992). The polyprotein is proteolytically processed by viral and host proteases into seven smaller proteins (Fig 2.1) denoted as P1, helper component (HC), P3, cylindrical inclusion (CI), nuclear inclusion A (NIa), nuclear inclusion B (NIb), capsid protein (CP), as well as two small putative proteins known as 6K1 and 6K2 (RIECHMANN *et al.*, 1992; LANGENBERG and ZHANG, 1997).

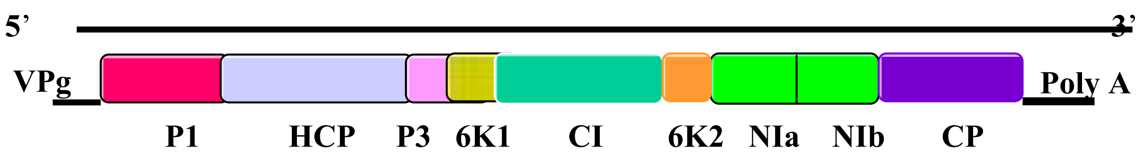

Fig. 1 The genome organisation of potyviruses (adapted from RIECHMANN *et al.*, 1992)

By now, the processing and functions of many of these proteins have been worked out, although for some, results are still controversial. On the other hand it is demonstrated, that many of them are multifunctional (VERCHOT and CARRINGTON, 1995; MAHAJAN *et al.,* 1996). Characteristically, all potyviruses induce different types of pinwheels or cylindrical cytoplasmic-inclusions in infected cells. These structures are aggregates of the viral encoded cylindrical inclusion protein (CI) and can be used as phenotypic criterion for assigning viruses to the genus potyvirus (EDWARDSON, 1992). Most potyviruses are transmitted in the non-persistent manner and epidemic levels of field spread often occur (SHUKLA *et al.*, 1994). In addition, members of this genus are also sap transmissible and quite some of them are spread by seeds (JOHANSEN *et al.*, 1994; SHUKLA *et al.,* 1994; BRUNT *et al.,* 1996).