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# Application of Antiviral Strategies Along with Mechanisms of Systemic Induced Resistance to Enhance the Defence Mechanism of Cowpea Plant Against Cowpea Mosaic Virus

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**ABSTRACT:** Cowpea mosaic virus (CPMV) is a non-enveloped plant virus of the comovirus group. Infection of a susceptible cowpea leaf causes a "mosaic" pattern in the leaf, and results in high virus yields (1-2 g/kg). Its genome consists of 2 molecules of positive-sense RNA (RNA-1 and RNA-2) which are separately encapsidated. Both RNA1 and RNA2 have a VPg (virus genome-linked protein) at the 5' end, and polyadenylation at the 3' end. Genomic RNA1 and RNA2 are expressed by a polyprotein processing strategy. RNA1 encodes helicase, VPg, protease and RdRp. RNA2 encodes movement protein and coat protein. The virus particles are 28 nm in diameter and contain 60 copies each of a Large (L) and Small (S) coat protein. The structure is well characterised to atomic resolution, and the viral particles are thermostable. The identification of the virus is attributed to Lister and Thresh in 1955,<sup>[1]</sup> but it is now known as a variant of the Sunn-hemp mosaic virus.

**KEYWORDS-**Cowpea mosaic virus, plant, defence, resistance, antiviral, systemic

## I. INTRODUCTION

CPMV displays a number of features that can be exploited for nanoscale biomaterial fabrication. Its genetic, biological and physical properties are well characterised, and it can be isolated readily from plants. There are many stable mutants already prepared that allow specific modification of the capsid surface. It is possible to attach a number of different chemicals to the virus surface<sup>[3][4]</sup> and to construct multilayer arrays of such nanoparticles on solid surfaces. This gives the natural or genetically engineered nanoparticles a range of properties which could be useful in nanotechnological applications such as biosensors, catalysis and nanoelectronic devices.[1,2,3]

One example use of CPMV particles is to amplify signals in microarray based sensors. In this application, the virus particles separate the fluorescent dyes used for signaling in order to prevent the formation of non-fluorescent dimers that act as quenchers.<sup>[5]</sup> Another example is the use of CPMV as a nanoscale breadboard for molecular electronics.<sup>[6]</sup>

CPMV particles have also shown potential for in-situ vaccination in cancer immunotherapy

The cowpea (*Vigna unguiculata*) is an annual herbaceous legume from the genus *Vigna*. Its tolerance for sandy soil and low rainfall have made it an important crop in the semiarid regions across Africa and Asia. It requires very few inputs, as the plant's root nodules are able to fix atmospheric nitrogen, making it a valuable crop for resource-poor farmers and well-suited to intercropping with other crops. The whole plant is used as forage for animals, with its use as cattle feed likely responsible for its name.

Four subspecies of cowpeas are recognised, of which three are cultivated. A high level of morphological diversity is found within the species with large variations in the size, shape, and structure of the plant. Cowpeas can be erect, semierect (trailing), or climbing. The crop is mainly grown for its seeds, which are high in protein, although the leaves and immature seed pods can also be consumed.

Cowpeas were domesticated in Africa<sup>[4]</sup> and are one of the oldest crops to be farmed. A second domestication event probably occurred in Asia, before they spread into Europe and the Americas. The seeds are usually cooked and made into stews and curries, or ground into flour or paste.[4,5,6]



Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world production. A 1997 estimate suggests that cowpeas are cultivated on 12.5 million hectares (31 million acres) of land, have a worldwide production of 3 million tonnes and are consumed by 200 million people on a daily basis.<sup>[5]</sup> Insect infestation is a major constraint to the production of cowpea, sometimes causing over 90% loss in yield.<sup>[6]</sup> The legume pod borer *Maruca vitrata* is the main preharvest pest of the cowpea and the cowpea weevil *Callosobruchus maculatus* the main postharvest pest.

Taxonomy and etymology

*Vigna unguiculata* is a member of the *Vigna* (peas and beans) genus. *Unguiculata* is Latin for "with a small claw", which reflects the small stalks on the flower petals.<sup>[7]</sup> Common names for cultivated cowpeas include black-eye pea,<sup>[8]</sup> southern pea,<sup>[9]</sup> niebe<sup>[10]</sup> (alternatively ñebbe),<sup>[11]</sup> and crowder pea.<sup>[12]</sup> All cultivated cowpeas are found within the universally accepted *V. unguiculata* subspecies *unguiculata* classification, which is then commonly divided into four cultivar groups: *unguiculata*, *biflora*, *sesquipedalis*, and *textilis*.<sup>[13][14]</sup> The classification of the wild relatives within *V. unguiculata* is more complicated, with over 20 different names having been used and between 3 and 10 subgroups described.<sup>[13][15]</sup> The original subgroups of *stenophylla*, *dekintiana*, and *tenuis* appear to be common in all taxonomic treatments, while the variations *pubescens* and *protractor* were raised to subspecies level by a 1993 characterisation.<sup>[13][16]</sup>

The first written reference of the word 'cowpea' appeared in 1798 in the United States.<sup>[7]</sup> The name was most likely acquired due to their use as a fodder crop for cows.<sup>[17]</sup> Black-eyed pea, a common name used for the *unguiculata* cultivar group, describes the presence of a distinctive black spot at the hilum of the seed. Black-eyed peas were first introduced to the southern states in the United States and some early varieties had peas squashed closely together in their pods, leading to the other common names of southern pea and crowder pea.<sup>[7]</sup>

The *sesquipedalis* subspecies arrived in the United States via Asia. It is characterised by unusually long pods, leading to the Latin name (*sesquipedalis* means "foot and a half long") and the common names of yardlong bean, asparagus bean, and Chinese long-bean.<sup>[18]</sup>

Common names of <i>Vigna unguiculata unguiculata</i> cultivar groups	
Group	Common name
Unguiculata	black-eyed pea, crowder-pea, southern pea, niebe, ñebbe
Biflora	catjang, sow-pea
Sesquipedalis	yardlong bean, asparagus bean, Chinese long-bean
Textilis	wild cowpea, African cowpea, Ethiopian cowpea

Description

A large morphological diversity is found within the crop, and the growth conditions and grower preferences for each variety vary from region to region.<sup>[13]</sup> However, as the plant is primarily self-pollinating, its genetic diversity within varieties is relatively low.<sup>[19]</sup> Cowpeas can either be short and bushy (as short as 20 cm or 8 in) or act like a vine by climbing supports or trailing along the ground (to a height of 2 m or 6 ft 7 in).<sup>[20][21]</sup> The taproot can penetrate to a depth of 2.4 m (7 ft 10 in) after eight weeks.<sup>[22]</sup>

The size and shape of the leaves vary greatly, making this an important feature for classifying and distinguishing cowpea varieties.<sup>[23]</sup> Another distinguishing feature of cowpeas is the long 20–50 cm (8–20 in) peduncles, which hold the flowers and seed pods. One peduncle can support four or more seed pods.<sup>[22]</sup> Flower colour varies through different shades of purple, pink, yellow, and white and blue.<sup>[21]</sup>

Seeds and seed pods from wild cowpeas are very small,<sup>[22]</sup> while cultivated varieties can have pods between 10 and 110 cm (4 and 43 in) long.<sup>[24]</sup> A pod can contain six to 13 seeds that are usually kidney-shaped, although the seeds become more spherical the more restricted they are within the pod.<sup>[20][22]</sup> Their texture and colour are very diverse.

They can have a smooth or rough coat and be speckled, mottled, or blotchy. Colours include white, cream, green, red, brown, and black, or various combinations.<sup>[22]</sup>

### History

Compared to most other important crops, little is known about the domestication, dispersal, and cultivation history of the cowpea.<sup>[25]</sup> Although there is no archaeological evidence for early cowpea cultivation, the centre of diversity of the cultivated cowpea is West Africa, leading an early consensus that this is the likely centre of origin and place of early domestication.<sup>[26]</sup> New research using molecular markers has suggested that domestication may have instead occurred in East Africa and currently both theories carry equal weight.<sup>[25]</sup>

While the date of cultivation began may be uncertain, it is still considered one of the oldest domesticated crops.<sup>[27]</sup> Remains of charred cowpeas from rock shelters in Central Ghana have been dated to the 2nd millennium BC.<sup>[28]</sup> In 2300 BC, the cowpea is believed to have made its way into Southeast Asia, where secondary domestication events may have occurred.<sup>[14]</sup> From there they traveled north to the Mediterranean, where they were used by the Greeks and Romans.<sup>[29]</sup> The first written references to the cowpea were in 300 BC and they probably reached Central and North America during the slave trade through the 17th to early 19th centuries.<sup>[14][26]</sup>

### Cultivation

Cowpeas thrive in poor dry conditions, growing well in soils up to 85% sand.<sup>[30]</sup> This makes them a particularly important crop in arid, semidesert regions where not many other crops will grow. As well as an important source of food for humans in poor, arid regions, the crop can also be used as feed for livestock.<sup>[31]</sup> Its nitrogen-fixing ability means that as well as functioning as a sole crop, [7,8,9] the cowpea can be effectively intercropped with sorghum, millet, maize, cassava, or cotton.<sup>[32]</sup>

The optimum temperature for cowpea growth is 30 °C (86 °F), making it only available as a summer crop for most of the world. It grows best in regions with an annual rainfall between 400 and 700 mm (16 and 28 in). The ideal soils are sandy and it has better tolerance for infertile and acid soil than most other crops. Generally, 133,000 seeds are planted per hectare (54,000/acre) for the erect varieties and 60,000 per hectare (24,000/acre) for the climbing and trailing varieties. The seeds can be harvested after about 100 days or the whole plant used as forage after about 120 days. Leaves can be picked from 4 weeks after planting.<sup>[33]</sup>

These characteristics, along with its low fertilisation requirements, make the cowpea an ideal crop for resource-poor farmers living in the Sahel region of West Africa. Early-maturing varieties of the crop can thrive in the semiarid climate, where rainfall is often less than 500 mm (20 in). The timing of planting is crucial, as the plant must mature during the seasonal rains.<sup>[34]</sup> The crop is mostly intercropped with pearl millet, and plants are selected that provide both food and fodder value instead of the more specialised varieties.<sup>[35]</sup>

Storage of the seeds can be problematic in Africa due to potential infestation by postharvest pests. Traditional methods of protecting stored grain include using the insecticidal properties of Neem extracts, mixing the grain with ash or sand, using vegetable oils, combining ash and oil into a soap solution or treating the cowpea pods with smoke or heat.<sup>[36]</sup> More modern methods include storage in airtight containers, using gamma irradiation, or heating or freezing the seeds.<sup>[37]</sup> Temperatures of 60 °C (140 °F) kill the weevil larvae, leading to a recent push to develop cheap forms of solar heating that can be used to treat stored grain.<sup>[38]</sup> One of the more recent developments is the use a cheap, reusable double-bagging system (called PICs) that asphyxiates the cowpea weevils.<sup>[39]</sup>

### Pests and diseases

Insects are a major factor in the low yields of African cowpea crops, and they affect each tissue component and developmental stage of the plant. In bad infestations, insect pressure is responsible for over 90% loss in yield.<sup>[6]</sup> The legume pod borer, *Maruca vitrata*, is the main preharvest pest of the cowpea.<sup>[40]</sup> Other important pests include pod sucking bugs, thrips, aphids, cowpea curculios and the post-harvest cowpea weevil, *Callosobruchus maculatus*.<sup>[6][41][42][43]</sup>

*M. vitrata* causes the most damage to the growing cowpea due to their large host range and cosmopolitan distribution.<sup>[44]</sup> It causes damage to the flower buds, flowers, and pods of the plant, with infestations resulting in a 20–



88% loss of yield.<sup>[44]</sup> While the insect can cause damage through all growth stages, most of the damage occurs during flowering.<sup>[44]</sup> Biological control has had limited success, so most preventive methods rely on the use of agrichemicals. Genetically modified cowpeas has been developed to express the cry protein from *Bacillus thuringiensis*, which is toxic to lepidopteran species including the maruca.<sup>[45]</sup> BT Cowpea was commercialised in Nigeria in 2019.<sup>[46]</sup>

Severe *C. maculatus* infestations can affect 100% of the stored peas and cause up to 60% loss within a few months.<sup>[47][48]</sup> The weevil generally enters the cowpea pod through holes before harvest and lays eggs on the dry seed.<sup>[49]</sup> The larvae burrow their way into the seed, feeding on the endosperm. The weevil develops into a sexually mature adult within the seed.<sup>[50]</sup> An individual bruchid can lay 20–40 eggs, and in optimal conditions, each egg can develop into a reproductively active adult in 3 weeks.<sup>[51]</sup> The most common methods of protection involve the use of insecticides, the main pesticides used being carbamates, synthetic pyrethroids, and organophosphates.<sup>[52]</sup>

Cowpea is susceptible to nematode, fungal, bacterial, and virus diseases, which can result in substantial loss in yield.<sup>[53]</sup> Common diseases include blights, root rot, wilt, powdery mildew, root knot, rust and leaf spot.<sup>[54]</sup> The plant is susceptible to mosaic viruses, which cause a green mosaic pattern to appear in the leaves.<sup>[54]</sup> The cowpea mosaic virus (CPMV), discovered in 1959, has become a useful research tool.<sup>[55]</sup> CPMV is stable and easy to propagate to a high yield, making it useful in vector development and protein expression systems.<sup>[55]</sup> One of the plant's defenses against some insect attacks is the cowpea trypsin inhibitor (CpTI).<sup>[56]</sup> CpTI has been transgenically inserted into other crops as a pest deterrent.<sup>[57]</sup> CpTI is the only gene obtained outside of *B. thuringiensis* that has been inserted into a commercially available genetically modified crop.<sup>[58]</sup>

Besides biotic stresses, cowpea also faces various challenges in different parts of the world such as drought, heat, and cold.<sup>[59]</sup> Drought lowers the growth rate and development, ultimately reducing yield, although cowpea is considered more drought tolerant than most other crops.<sup>[60]</sup> Drought at the preflowering stage in cowpea can reduce the yield potential by 360 kg/ha.<sup>[61]</sup> Crop wild relatives are the prominent source of genetic material, which can be tapped to improve biotic/abiotic tolerance in crops.<sup>[62]</sup> International Institute of Tropical Agriculture (IITA), Nigeria and Institut de l'Environnement et de Recherches Agricoles are looking to tap into the genetic diversity of wild cowpeas and transfer that into cultivars to make them more tolerant to different stresses and adaptive to climate change.<sup>[63]</sup>

### Culinary use

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Cowpeas are grown mostly for their edible beans, although the leaves, green seeds and pods can also be consumed, meaning the cowpea can be used as a food source before the dried peas are harvested.<sup>[64]</sup> Like other legumes, cowpeas are cooked to make them edible, usually by boiling.<sup>[65]</sup> Cowpeas can be prepared in stews, soups, purees, casseroles and curries.<sup>[66][67]</sup> They can also be processed into a paste or flour.<sup>[68]</sup> Chinese long beans can be eaten raw or cooked, but as they easily become waterlogged are usually sautéed, stir-fried, or deep-fried.<sup>[69]</sup>

A common snack in Africa is koki or moin-moin, where the cowpeas are mashed into a paste, mixed with spices and steamed in banana leaves.<sup>[70]</sup> They also use the cowpea paste as a supplement in infant formula when weaning babies off milk.<sup>[71]</sup> Slaves brought to America and the West Indies cooked cowpeas much the same way as they did in Africa, although many people in the American South considered cowpeas not suitable for human consumption.<sup>[72]</sup> A popular dish was Hoppin' John, which contained black-eyed peas cooked with rice and seasoned with pork. Over time, cowpeas became more universally accepted and now Hoppin'[10,11,12] John is seen as a traditional Southern dish ritually served on New Year's Day.<sup>[73]</sup>

### Nutrition and health

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Cowpea seeds provide a rich source of proteins and food energy, as well as minerals and vitamins.<sup>[68]</sup> This complements the mainly cereal diet in countries that grow cowpeas as a major food crop.<sup>[74]</sup> A seed can consist of 25% protein and has very low fat content.<sup>[75]</sup> Cowpea starch is digested more slowly than the starch from cereals, which is more beneficial to human health.<sup>[68]</sup> The grain is a rich source of folic acid, an important vitamin that helps prevent neural tube defects in unborn babies.<sup>[76]</sup>

The cowpea has often been referred to as "poor man's meat" due to the high levels of protein found in the seeds and leaves.<sup>[65]</sup> However, it does contain some antinutritional elements, notable phytic acid and protease inhibitors, which reduce the nutritional value of the crop.<sup>[68]</sup> Methods such as fermentation, soaking, germination, debranning, and



autoclaving are used to combat the antinutritional properties of the cowpea by increasing the bioavailability of nutrients within the crop.<sup>[77]</sup> Although little research has been conducted on the nutritional value of the leaves and immature pods, what is available suggests that the leaves have a similar nutritional value to black nightshade and sweet potato leaves, while the green pods have less antinutritional factors than the dried seeds.<sup>[68]</sup>

Production and consumption

Cow peas, dry production, 2020 <sup>[78]</sup>	
Country	Weight (tonnes)
Nigeria	3,647,115
Niger	2,637,486
Burkina Faso	666,023
Kenya	264,160
Senegal	253,897
All others	1,447,422
World	8,916,103

Most cowpeas are grown on the African continent, particularly in Nigeria and Niger, which account for 66% of world cowpea production.<sup>[79]</sup> The Sahel region also contains other major producers such as Burkina Faso, Ghana, Senegal, and Mali. Niger is the main exporter of cowpeas and Nigeria the main importer. Exact figures for cowpea production are hard to come up with as it is not a major export crop. Estimating world cowpea production is rather difficult, as it is usually grown in a mixture with other crops, but according to a 1997 estimate, cowpeas were cultivated on 12.5 million hectares (31 million acres) and had a worldwide production of 3 million metric tons (3,000,000 long tons; 3,300,000 short tons).<sup>[5]</sup> While they play a key role in subsistence farming and livestock fodder, the cowpea is also seen as a major cash crop by Central and West African farmers, with an estimated 200 million people consuming cowpea on a daily basis.<sup>[80]</sup>

According to the Food and Agriculture Organization of the United Nations, as of 2012, the average cowpea yield in Western Africa was an estimated 483 kilograms per hectare (431 lb/acre),<sup>[79]</sup> which is still 50% below the estimated potential production yield.<sup>[81]</sup> In some tradition cropping methods, the yield can be as low as 100 kilograms per hectare (89 lb/acre).<sup>[21]</sup>

Outside Africa, the major production areas are Asia, Central America, and South America. Brazil is the world's second-leading producer of cowpea seed, accounting for 17% of annual cowpea production, although most is consumed within the country.<sup>[4]</sup>

II. DISCUSSION

Cowpea [*Vigna unguiculata*(L.) Walp.] seedlings, raised from seeds treated with acibenzolar-S-methyl [benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester; BTH], were inoculated at 7 days old with *Colletotrichum destructivum*. Tissue penetration was reduced markedly and intracellular infection vesicles were invariably restricted to the initially-infected epidermal cells of treated hypocotyls and leaves. The destructive necrotrophic phase of disease development was effectively blocked by a hypersensitive response in these cells, thereby protecting seedlings against damping-off. The enhanced resistance of BTH-treated tissues was associated with rapid, transient increases in the activities of two key enzymes of the phenylpropanoid/flavonoid pathway, phenylalanine ammonia-lyase (PAL) and chalcone isomerase (CHI). Subsequently, there was an early, accelerated accumulation of the isoflavonoid phytoalexins kievitone and phaseollidin in treated hypocotyls. In addition, several protein bands, in the low-molecular weight range, developed in these treated challenged tissues. These responses occurred following inoculation of a normally susceptible cultivar (IT82E-60) with the pathogen, and were not observed in induced, uninoculated tissues. These results suggest that BTH protects cowpea seedlings by potentiating an early defence response rather than by altering the constitutive resistance of tissues.<sup>[13,14,15]</sup>

Cowpea miRNAs and Argonaute genes showed differential expression patterns in response to CPSMV challenge. Several biotic stresses affect cowpea production and yield. CPSMV stands out for causing severe negative impacts on cowpea. Plants have two main induced immune systems. In the basal system (PTI, PAMP-triggered immunity), plants recognize and respond to conserved molecular patterns associated with pathogens (PAMPs). The second type (ETI, Effector-triggered immunity) is induced after plant recognition of specific factors from pathogens. RNA silencing is another important defense mechanism in plants. Our research group has been using biochemical and proteomic approaches to learn which proteins and pathways are involved and could explain why some cowpea genotypes are resistant whereas others are susceptible to CPSMV. This current study was conducted to determine the role of cowpea miRNA in the interaction between a resistant cowpea genotype (BRS-Marataoã) and CPSMV. Previously identified and deposited plant microRNA sequences were used to find out all possible microRNAs in the cowpea genome. This search detected 617 mature microRNAs, which were distributed in 89 microRNA families. Next, 4 out of these 617 miRNAs and their possible target genes that encode the proteins Kat-p80, DEAD-Box, GST, and SPB9, all involved in the defense response of cowpea to CPSMV, had their expression compared between cowpea leaves uninoculated and inoculated with CPSMV. Additionally, the differential expression of genes that encode the Argonaute (AGO) proteins 1, 2, 4, 6, and 10 is reported. In summary, the studied miRNAs and AGO 2 and AGO4 associated genes showed differential expression patterns in response to CPSMV challenge, which indicate their role in cowpea defense.[16,17,18]

### III. RESULTS

Systemic virus infection of plants involves; intracellular replication, cell-to-cell movement within the inoculated leaf, and subsequently, long-distance spread to other plant parts via the vasculature (vascular movement). Cell-to-cell movement occurs through the plasmodesma (PD), which are regulated channels in the cell wall connecting adjacent cells. These PD are modified by plant viral movement proteins (MPs) to allow passage of a viral RNA-MP complex as happens with Tobacco mosaic virus (TMV), or virions as happens with Cowpea mosaic virus (CPMV). With the latter virus, virions move through tubules built-up from the MP (tubule-guided cell-to-cell movement). For vascular movement, viruses must enter (loading), translocate through, and exit (unloading) from the phloem. Phloem (un)loading occurs through specialized PD, named the pore-plasmodesma-unit (PPU), connecting the companion cell (CC) and sieve element (SE). The PPU allows passage of much larger molecules than mesophyll PD do. Because of the peculiarities inherent to phloem tissue (e.g. PPU), mechanisms of cell-to-cell movement are usually distinct from those of vascular movement the same virus. For instance, TMV requires the viral coat protein (CP) for transport of virions through PPU, but the CP is dispensable for cell-to-cell movement. The success of plant virus infection is also the consequence of an antagonistic balance between viral infection and plant host defence mechanisms that specifically target viral replication (e.g. RNA silencing), or movement (e.g. systemic acquired resistance). In this research thesis CPMV was used as a model for investigations on the mechanisms of systemic infection of plants. Since CPMV replication and cell-to-cell movement are well-investigated, the thesis research was concentrated on vascular movement of CPMV and on barriers imposed by different plant species against systemic infection by this virus. To examine the characteristics of vascular movement in *Vigna unguiculata* (cowpea), GFP-expressing CPMV (CPMV-GFP) was mechanically inoculated to primary leaves and infection was followed over time. CPMV-GFP was loaded into both major and minor veins of the primary leaves and unloaded exclusively from major veins, preferentially class III, in the secondary leaves similar to the route of photo-assimilates via phloem. Using electron microscopy, virus infection was observed in all vascular cell types of the loading and unloading sites, with the exception of CC and SE. Furthermore, tubules transporting virions were never found in the PD connecting; phloem parenchyma cells (PPC) and CC, or CC and SE (i.e. PPU). Since in cowpea the SE is symplasmically connected only to the CC, these observations suggest that, unlike cell-to-cell movement, CPMV vascular movement is not tubule-guided. Mutational analysis by reverse genetics is the most common approach to the study of viral factors necessary for vascular movement. CPMV requires its MP and both coat proteins (CPs) for tubule-guided cell-to-cell movement, deletion of any of these genes results in impeded local spread and this restriction severely hampers application of reverse genetics on CPMV for this purpose. An attempt was made to circumvent this problem by providing the CPs intrans by agroinfiltration in *N. benthamiana* to complement cell-to-cell movement of a CPMV mutant devoid of CPs (CPMV-DCP). The aim was to observe whether the mutant would exit from vascular tissue in the absence of CPs in the upper leaves. While trans complementation of CPMV-DCP cell-to-cell movement was demonstrated in planta, the extent of spread was not sufficient to allow CPMV-DCP phloem loading, thus the phloem unloading of the mutant within the upper leaves could not be analysed. Immunoblot analysis of vascular sap from infected cowpea plants showed the presence solely of viral CPs. Furthermore, virions were found in the vasculature of CPMV-immune cowpea scions grafted on CPMV-inoculated susceptible rootstocks. These results indicate that CPMV circulates in the vasculature in form of mature virions.

However, it could not be unequivocally determined whether virions were located in the phloem or in the xylem. As systemic spread by xylem has been reported for beetle transmissible viruses like CPMV, beetle transmission was mimicked by gross-wound inoculation. However, in this case, as with mechanical inoculation using an abrasive, CPMV spread systemically via the phloem, i.e. directed to sink-leaves solely like the flow of photo-assimilates. This confirms that phloem is the prevailing route for CPMV vascular movement. The potential role of RNA silencing during establishment of infection by CPMV was studied. Using GFP-expressing CPMV constructs and *N. benthamiana* as host, the number of infection foci was recorded in the absence or presence of different viral suppressors of RNA silencing, i.e. potyviral HC-Pro, tospoviral NSs and cucumoviral 2b. Upon inoculation with CPMV in vitro transcripts, HC-Pro and NSs, but not 2b, significantly increased the number of CPMV primary infection foci. These results indicate that RNA silencing already has an impact on the establishment of infection even at an early stage. Interestingly, the stimulating effect of suppressors was not observed upon inoculation with virions. This effect may be explained by the recent finding (Lomonosoff, personal communication) that the small (S) CP acts as a suppressor of RNA silencing. To assess the effect of RNA silencing on viral local spread, GFP-expressing CPMV constructs impaired in local spread were tested in the presence or absence of HC-Pro or NSs. Neither of these proteins affected the progress of infection, indicating that RNA silencing does not play a major role in this stage. *N. tabacum*, a semi-permissive host of CPMV, was used to further unravel the viral systemic infection process. CPMV does not infect *N. tabacum* systemically despite extensive local spread in inoculated leaves. It is shown that neither incubation temperature nor RNA silencing-, salicylic acid- or ethylene-mediated resistance mechanisms are the limiting factor for CPMV systemic infection. Although CPMV-infected *N. tabacum* plants are normally asymptomatic, symptoms (i.e. necrotic lesions) in the inoculated leaves were observed at low (15 °C) temperature, but not systemic movement. Grafting experiments indicate that CPMV is not capable of phloem loading in *N. tabacum*, a finding that makes this plant species an interesting system for investigations of the host factors involved in CPMV vascular movement. Finally, possible mechanisms of vascular movement of CPMV are presented based on the results obtained in this thesis. Moreover, the various virus-host interactions, which contribute to the success or failure of systemic infection, are put into perspective. [19,20,21,22]

#### IV. CONCLUSION

Invasion of cowpea primary leaves occurred preferentially through the leaf epidermal cells by penetration tubes that emerged from appressoria. The penetration tube swelled within 48 h to form a spherical infection vesicle inside the epidermal cell in a biotrophic interaction. Subsequently, it became multilobed and multiseptated. By 4 d after inoculation, one or more large primary hyphae emerged from the lateral lobes and grew intracellularly and further colonized several adjacent epidermal host cells, characterizing the necrotrophic phase of infection. Primary leaves of the resistant cowpea genotype, TE 97-411, showed enhanced penetration resistance. [23]

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