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Research Application Summary

Testing the efficacy of artificial microRNAs to control cassava brown streak disease

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Abstract

Cassava brown streak disease (CBSD) is a major constraint to cassava production in East Africa. It is caused by at least two single stranded (+) RNA viruses (CBSV and CBSUV). To date, only a few genetic sources of resistance to the disease in cassava are known. Artificial microRNAs (amiRNAs) have in the recent been employed to control plant viruses. In this study, 21 nt of the *A. thaliana* pre-mi159a were replaced with 21 nt conserved sequences selected across the CBSV and CBSUV genomes, generating 11 amiRNA constructs targeting different CBSV genes (P1 {CBSV-Ug and CBSV-Tz}, P3, CI, NIb, CP and 3'UTR).The modified precursors were then sub-cloned in a shuttle vector CGT11003-I and subsequently cloned into a binary vector AKK1420 with an RNAi cassette targeting the green fluorescent protein (GFP), as an internal silencing control. Transient studies of these amiRNA constructs in transgenic *N. benthamiana* (16C) using *Agrobacterium* (GV3001 strain), showed that constructs targeting CBSV-P1, CBSUV-P1, NIb, CP and UTR expressed miRNAs specific to their target nucleotides of CBSUV. Transient protection studies showed varied levels of resistance to the homologous virus roughly corresponding to the level of siRNA accumulation using Northern analysis. Artificial microRNAs (amiRNA) therefore have the potential to control the spread of CBSUV.

Key words: Artificial microRNAs, cassava brown streak disease virus resistance

Résumé

La maladie brune de strie de manioc (CBSD) est une contrainte importante à la production de manioc en Afrique Orientale. Elle est provoquée par au moins deux virus simples ratés d'ARN(+) : CBSV et CBSUV. Jusqu'aujourd'hui, seulement quelques sources génétiques de résistance à la maladie dans le manioc sont connues. Des microARNs artificiels (amiRNAs)

ont été utilisés récemment pour contrôler des virus des plantes. Dans cette étude, 21 nt de *A. thaliana* pre-mi159a ont été remplacés par 21 nt ordres conservés choisis parmi les génomes CBSV et CBSUV, engendrant 11 concepts d'amiRNA visant les différents gènes de CBSV (P1 {CBSV-Ug et CBSV-Tz}, P3, CI, NIb, CP et 3'UTR). Les précurseurs modifiés ont été alors sous-clonés dans un vecteur de navette CGT11003-I et plus tard clonés dans un vecteur binaire AKK1420 avec une cassette de ARNi visant la protéine fluorescente verte (GFP), comme un contrôle d'amortissement interne. Les études passagères de ces concepts d'amiRNA dans *N. benthamiana* (16C) transgénique, employant l' *Agrobacterium* (tension GV3001), ont prouvé que les concepts visant CBSV-P1, CBSUV-P1, NIb, CP et UTR ont exprimé des miRNAs spécifiques à leurs nucléotides de cible de CBSUV. Les études transitoires de protection ont montré des niveaux variés de résistance au virus homologue correspondant rudement au niveau de l'accumulation de siRNA en utilisant l'analyse de Northern. Les microARNs artificiels (amiRNA) ont donc le pouvoir de contrôler la propagation de CBSUV.

Mots clés: MicroARNs artificiels, résistance du virus de la maladie brune de strie de manioc

Background

Cassava *(Manihot esculenta* Crantz) production in East, Central and Southern Africa is greatly constrained by viral diseases (Were *et al.*, 2002). Cassava Brown Streak Disease (CBSD), a re-emerging disease, is a major threat to cassava productivity because it also affects the recently improved varieties resistant to cassava mosaic disease. CBSD in Uganda was first reported in 1945 on cassava lines imported from Tanzania, to control the CMD epidemic of the 1930s and 1940s (Alicai *et al.*, 2007). The affected crops were destroyed and the disease contained, until 2004 when it re-emerged in central Uganda. The disease is now considered the primary threat to cassava production (Alicai *et al.*, 2007).

The causal agent of CBSD, the cassava brown streak virus (CBSV) was identified to be an *Ipomovirus* (family *Potyviridae*) (Monger *et al.*, 2001). Based on partial sequencing of the viral coat protein gene (~25% of the gene; only 220 bases) of the virus, Alicai *et al.*, (2007) reported the likelihood of presence of variant species, that may have resulted in the re-emergence of the disease in Uganda. More recent studies based on the coat protein-encoding sequences (1,101 nucleotides)

indicated that two geographically distinct strains exist; one from the Lake Victoria basin and another from the Indian Ocean coastal region (Mbanzibwa *et al.,* 2009a). Studies also indicate that the whitefly, *Bemisia tabaci* Gennadius transmits CBSV from infected to healthy plants (Maruthi *et al.,* 2005). The management of CBSD is currently managed by planting disease free stakes. Unfortunately, this has not been useful because the vector is difficult to control. In addition, it is increasingly getting difficult to find disease free planting materials. This leaves us with only one viable option, i.e., the use of resistance. This study was carried out to test the potential of microRNAs (amiRNAs) to control CBSD.

Literature Summary

Resistance to viruses can be mediated through RNA silencing (Prins *et al.,* 2008). Expression of translatable or untranslatable RNA and antisense RNA corresponding to the coat protein, in two viruses of *portyviridae* has resulted in resistance when tested in transgenic tobacco via sap inoculation. It is known that expression of transgenes that have homologous sequences to viral sequences often lead to post-transcriptional gene silencing. Pathogen derived resistance (PDR), has therefore often been utilised in crops to generate resistance to viruses (Vanderschuren *et al.,* 2007). PDR strategies can be roughly divided into two groups; those that involve the production of transgenic protein e.g., coat protein (CP), replicase and movement protein (MP) and those that function at RNA level (e.g. sense, anti-sense, ribozyme, dsRNA). It is known that expression of double stranded (RNA) in plant cells can trigger silencing of transgenes, endogens, and invasive viruses in a sequence specific manner (Vanderschuren *et al.,* 2007), a phenomenon referred to as RNA interference (RNAi). This phenomenon has been used to create resistance against cucumber mosaic virus in tobacco (Kalantidis *et al*., 2002) and against barley yellow dwarf virus (BYDV) in barley (Wang *et al.,* 2000).

Study Description

More recently, in addition to RNA interference (RNAi), artificial microRNAs (amiRNAs) have been employed to control plant viruses. microRNAs (MiRNA) are 20-24 nt long non-coding RNAs, highly conserved across plant and animal species and expressed as precursors of ~263 nt, that are further spliced to yield ~21 nt miRNAs involved in gene regulation and development. In this study, 21 nt of the *Arabidopsis thaliana* (pre-mi159a) were replaced with 21 nt conserved sequences selected across the CBSV and CBSUV genomes, generating

11 amiRNA constructs targeting different CBSV genes (P1 {CBSV-Ug and CBSV-Tz}, P3, CI, NIb, CP and 3'UTR).The modified precursors were then sub-cloned in a shuttle vector CGT11003-I and subsequently cloned into a binary vector AKK1420 with an RNAi cassette targeting the green fluorescent protein (GFP), as an internal silencing control. Transient studies of these amiRNA constructs in transgenic *Nicotiana benthamiana* (16C) using *Agrobacterium* (GV3001 strain), showed that constructs targeting CBSV-P1, CBSUV-P1, NIb, CP and UTR expressed miRNAs specific to their target nucleotides of CBSUV. Transient protection studies showed varied levels of resistance to the homologous virus roughly corresponding to the level of siRNA accumulation using Northern analysis. Artificial microRNAs (amiRNA) therefore have the potential to control the spread of CBSUV, providing an alternative method to control the virus in cassava crop plants.

Recommendation

Artificial microRNAs (amiRNA) have the potential to control the spread of Cassava Brown Streak Uganda Virus. The designed amiRNA constructs will therefore be used to transform *N. benthamiana* model plants and cassava crop plant to establish their efficacy to control CBSD in cassava as an alternative method.

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