FEDERAL REPUBLIC OF NIGERIA

Application Form for a Confined Field Trial

This form should be forwarded to **Director General/Chief Executive Officer/, National Biosafety Management Agency** on completion.

This application form consists of seven parts:

1. Administrative information
2. Plant information
3. Trial Description
4. Genetic Confinement
5. Material Confinements
6. Records, Personnel, and Planning
7. Declaration

1. Administrative information

Purpose of Application:
[Application for a confined field trial for (name of Crop species and introduced trait).]

The transgenic cassava lines to be tested were produced to increase the starch yield of storage roots, i.e. to generate plants with more and/or bigger storage roots with higher starch content per storage root dry weight. The constructs developed for plant transformation target key mechanisms to increase source- and sink capacities, and by bolstering sugar accumulation, sugar transport and starch accumulation, we expect to obtain an increase in storage root starch yield.
All constructs were developed at Friedrich-Alexander-University (FAU) Erlangen-Nuremberg (Germany) in the laboratory of Prof. Dr. Uwe Sonnewald and transformed into cassava genotype 60444 in the laboratory of Prof. Dr. Wilhelm Gruissem at Eidgenössische Technische Hochschule (ETH) Zurich (Switzerland).

Previous Applications or Approvals:
[Information on the status of this crop and trait, including pending, approved, or denied applications for field trials and commercial releases here or in other jurisdictions. Indicate also if this is a new application or a renewal.]

This is a new application as it involves cassava clones transformed for increased starch content in the roots. No application has been previously made for CFT of the below-described transgenic cassava clones and commercial release of these transgenic cassava clones before in any jurisdiction.
In 2017, the applicant, International Institute of Tropical Agriculture (IITA), received permit for confined field trial (NBMA/CFT/005) to test transgenic cassava other than those included in the present

application to be conducted in the below-mentioned confined field site.

Applicant:
[Name of applying institution, which may also include the name of the Principal Investigator or other key personnel.]

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Proposed Location and Size of Trial:
[Name, address, e-mail, phone, and facsimile of the Trial Manager as well as GPS information or description of the exact location and size of the trial site (attach sketch map).]

Name of Trial Manager: Anna Vittoria Carluccio
Address: IITA Headquarters, PMB 5320, Oyo Road, Ibadan 200001, Oyo State
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GPS information on location of the Confined Field Trial (CFT); Geographical coordinates: 7.490940 N – 3.903835 E.
See Annex A for an aerial image of the confined field site.

Size: The confined field site is about 2900 square meter (31m x 93m) large. The trial will be conducted on a smaller area within screen-houses, one of which already exist and two more will be buit upon approval of this application.

Proposed Duration of Trial: 36 months

Expected starting date: December 2018

Expected termination date: December 2021

2. Plant Information

2.1 Unmodified Plant Information

This section describes the characteristics of the unmodified plant as it relates to confinement. Important information pertains to the plant's reproductive mechanisms and its ability to escape, establish, and persist in the environment into which it is being introduced.

Plant Species Name (Common and Scientific):

Cassava (*Manihot esculenta*, Crantz)

Center of Origin:
[What is the center of origin of the unmodified plant?]

Cassava originates from South America. The unmodified cassava cultivar 60444 included in this study has been selected in IITA from West-African landraces.

Reproductive Mechanism of the Plant:
[Describe the reproductive biology of the plant. This information may be obtained from Organization for Economic Co-operation and Development (OECD), biology consensus documents or similar sources, and should include relevant information on: inter- and intra-specific breeding; pollen production, dispersal and viability; seed production and dispersal; see dormancy, capacity for vegetative reproduction.]

Cassava is an open pollinated monoecious plant with male and female flowers borne separately on the same plant.
Male and female flowers are borne on the same branched panicle, with female flowers at the base and male flowers toward the tip. In a given inflorescence, female flowers open from one to a few weeks earlier than the male flowers. By the time male flowers open, the female flowers on the same branch have been fertilized or have aborted. However, because flowering on a single plant may last for more than 2 months, pollen from a flower may fertilize other flowers on the same plant, or flowers on surrounding plants, with the proportion of each dependent on the genotype, the environment, and the presence of pollinating insects.
The pollen grains of cassava are relatively large in size, and are sticky. Therefore, wind pollination appears to be of little consequence whereas several species of honeybees (*Apis mellifera*) are considered the main pollinators in Africa. Cassava pollen loses viability rapidly after it is shed.
Pollen viability seems to decline substantially after this time. In practice, breeders take care to perform pollinations within 1 h after collection of pollen to help ensure successful fertilization. Cassava can cross to some of its wild relatives e.g. *M. glaziovii, M. dicothoma, M. oligantha subsp.* Nesteli, which are not commonly found outside of South America.
Cassava seeds develop within small fruits, usually three per fruit, with 1-3 seeds being fertile (Jennings and Iglesias, 2002). Developing seeds are viable 2 months after pollination, and the fruit becomes

mature about 1 month after that, or about 3 months after pollination. Fruit dehiscence is explosive; the seed initially falls close to the mother plant but then may be further dispersed by ants. Newly harvested seeds exhibit physiological dormancy and require 3 to 6 months of storage at ambient temperature before they will germinate. Seed germination is favored by dry heat and complete darkness.
To conserve the positive attributes of known genotypes, cassava is normally vegetatively propagated by means of stem cuttings, which are known horticulturally as 'stakes'. Stakes are typically at least 20 cm long, and have 4 to 5 nodes each with a viable bud.

Tendency and Weediness:
[Is the unmodified plant regarded by agricultural experts as a weed in regions where it is cultivated? If so, are control methods available that may be used to effectively limit the dispersal and establishment of the unmodified plants? NOTE: The information on the confined field trial location and how the genetically modified plant will be managed are described elsewhere in this application.]

Cassava is neither invasive nor weedy as seed production is usually very limited.

Toxicity and Allergenicity:
[Is the plant species known to be a source of substances that are toxic or allergenic to humans or animals? If yes, identify the substances and levels that induce toxicity or allergenicity and the affected species.]

Cassava is one of 3,000 plant species that produce cyanogenic compounds that upon breakdown release hydrogen cyanide (HCN) and can therefore be toxic to humans (McMahon et al., 1995).
The main cyanogenic glucosides are linamarin (>90%) and lostraulin (<10%). Cassava cultivars have been classified into groups according to their cyanogenic glucoside content, measured as HCN equivalents or as taste of the roots (McMahon et al., 1995). In the cassava breeding program at IITA 100mg HCN equivalent per kg is the upper limit for a cultivar to be classified as low in cyanogenic glucosides. The cultivar 60444 averages up to 110mg HCN equivalent per kg and is therefore classified as 'slightly bitter'. Consumption of this cultivar thus needs prior processing to remove the cyanogens. However, as no cassava from the experimental field will be consumed, this potential danger does not come into effect. All plants will be incinerated within the confined field site.

2.2 Modified Plant Information

This section is intended to provide information on known or intended effects of the genetic modification or introduced trait that may affect confinement measures employed in the confined trial.

Describe the Intended Phenotypic Changes to the Plant

The intended phenotypic change to the transgenic cassava lines is an increased starch yield of storage roots, i.e. we intend to generate plants with bigger or more storage roots per plant and/or more starch content per storage root on dry weight basis. This phenotypic change should be achieved by simultaneously increasing source and sink capacities. The respective changes should be achieved by simultaneous alteration of expression of some of the following genes using constructs described below.

GPT – Glucose 6-phosphate/phosphate translocator of pea (*Pisum sativum*). GPTs transport glucose 6-phosphate in counter-exchange with phosphate or triose phosphate across inner envelope membranes of plastids. Overexpressing the GPT mainly in storage roots should increase the import of carbon skeletons (i.e. glucose 6-phosphate) into amyloplasts, the cell organelles responsible for the synthesis and storage of starch granules, through the polymerization of glucose. These carbon skeletons are needed as precursor for starch synthesis (Zhang et al., 2008; Jonik et al., 2012).
NTT – Nucleoside triphosphate translocator1 of thale cress (*Arabidopsis thaliana*). NTTs transport ATP in counter-exchange with ADP and Pi across inner envelope membranes of plastids. Overexpressing the NTT mainly in storage roots should increase the import of energy (in the form of ATP) into amyloplasts. The energy is needed for starch synthesis (Tjaden et al., 1998; Zhang et al., 2008; Jonik et al., 2012).
TMT – Tonoplast monosaccharide transporter1 of thale cress (*Arabidopsis thaliana*). TMTs transport monosaccharides in antiport with protons across the tonoplast membrane. Overexpressing the TMT mainly in leaves should increase the export of hexoses from the cytosol (into the vacuole). Lowering the cytosolic hexose content has been shown to alleviate down-regulation of photosynthetic genes and should thus increase production of photosynthesis products (Wingenter et al., 2010).
GlyDH – Glycolate dehydrogenase fusion protein of subunit D, E and F of *Escherichia coli*. GlyDH oxidizes glycolate to glyoxylate. Overexpressing the GlyDH targeted to chloroplasts mainly in leaves should prevent photorespiration by blocking an initial step of this metabolic pathway, the export of glycolate from chloroplasts (Nölke et al., 2014; Dalal et al., 2015).
RCase – RubisCO activase. A synthetic chimeric gene consisting of Arabidopsis and tobacco sequences is used. Consuming ATP, RCase removes products of RubisCO activity from RubisCO which maintains RubisCO activity. Overexpression of RCase mainly in leaves is supposed to increase the activation state of RubisCO, mainly under conditions of elevated temperature (Carmo-Silva et al., 2015).
OEP7-HxK – Outer envelope protein7 of thale cress (*Arabidopsis thaliana*) fused to yeast (*Saccharomyces cerevisiae*) hexokinase2. Hexokinases phosphorylate hexoses to hexose 6-phosphates consuming ATP and releasing ADP. Overexpression of a hexokinase at the outer envelope of plastids mainly in storage roots should lead to an increased production of glucose 6-phosphate in the vicinity of GPT and thus to a higher efficient uptake of carbon skeletons into amyloplasts fueling starch formation.

References

Carmo-Silva E, Scales JC, Madgwick PJ, Parry MAJ (2015). Optimizing RubisCO and its regulation for greater resource use efficiency. *Plant Cell Environ* 38, 1817-32.
Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu R, Sederoff HW (2015). A photorespiratory bypass increases plant growth and seed yield in the biofuel crop *Camelina sativa*. *Biotechnol for Biof* 8, 175.
Jonik C, Sonnewald U, Hajirezaei M-R, Flügge U-I, Ludewig F (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnol J* 10, 1088-98.
Nölke G, Houdelet M, Kreuzaler F, Peterhänsel C, Schillberg S (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (*Solanum tuberosum*) plastids strongly enhances photosynthesis and tuber yield. *Plant Biotechnol J* 12, 734-42.
Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998). Altered plastidic

ATP/ADP-translocator activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant J* 16, 531-40.
Wingenter K, Schulz A, Wormit A, Wic S, Trentmann O, Hoermiller II, Heyer AG, Marten I, Hedrich R, Neuhaus HE (2010). Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in Arabidopsis. *Plant Physiol* 154, 665-77.
Zhang L, Häusler RE, Greiten C, Hajirezaei M-R, Haferkamp I, Neuhaus HE, Flügge U-I, Ludewig F (2008). Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol J* 6, 453-64.

Intended Reproductive Effects:
[Does the genetic modification intentionally alter the reproductive biology of the plant? How do these changes affect strategies for confinement?

There is no intended reproductive effect on the transgenic plants. However, as it is intended to alter the source and sink capacities, an effect on flowering and/or on regrowth of plants from stakes cannot be excluded. Both, flowers and stakes are sink organs that compete with other sink organs, e.g. the storage roots, for photosynthate. While there is no indication that the reproduction mechanism of the transgenic lines could be different from control plants, if any change would occur, they would likely not be in favor of flower production and/or of generation of buds from stakes. Therefore, these changes are not likely to interfere with strategies for confinement.

What is the source of the genetic material? Is the source of the genetic material likely to affect the safe conduct of a confined field trial? If yes, how?
[Describe any known or intended introduction of infectious agents, plant, animal or human pathogens or allergens or toxins.]

The cassava cultivar 60444 used for transformation originated from Nigeria.
Other genetic material derived from other plants, plant viruses, bacteria or of synthetic origin:

<u>Plant sources:</u>

- *Manihot esculenta – Granule Bound Starch Synthase1* (*MeGBSS1*)
- *Solanum tuberosum – Soluble Starch Synthase3* (*SSS3*) promoter, *Leaf Specific1* (*LS1*) promoter, *Starch Phosphorylase* (*STP*) promoter, *H4* 3'-UTR and terminator
- *Arabidopsis thaliana – Chlorophyll a/b binding protein1* (*CAB1*) promoter, *Rubisco Small Subunit2B* (*RbcS2B*) promoter, *Rubisco Small Subunit3B* (*RbcS3B*) promoter, *Nucleoside Triphosphate Translocator1* (*NTT1*) coding sequence, *Tonoplast Monosaccharide Transporter1* (*TMT1*) coding sequence, 5´UTR-CDS *Outer Envelope Protein7* (*OEP7*), Chimeric *Rubisco activase* (*RCase*)
- *Solanum lycopersicum* – 3'-UTR-terminator *ATPase*
- *Pisum sativum – Glucose 6-phosphate/Phosphate Translocator* (*PsGPT*)
- *Nicotiana tabacum* – Chloroplast transit peptide from *RbcS41*, Chimeric *Rubisco activase* (*RCase*)

<u>Fungal sources:</u>

- *Saccharomyces cerevisiae* – Hexokinase2 (HxK2)

<u>Bacterial sources:</u>

- *Escherichia coli* – *Glycolate dehydrogenase* fusion of subunits DEF, *Hygromycin Phosphotransferase 2* (*hpt2*)
- *Agrobacterium tumefaciens* – 3´UTR-terminator from *Octopine Synthase*, 3´UTR-terminator from *Mannopine Synthase*, 3´UTR-terminator from *Gene7*, *Nopaline Synthase* promotor

Viral sources:
- *Barley Stripe Mosaic Virus* – *BSMV* 5'-UTR
- *Cauliflower Mosaic Virus* – 35S terminator
- *Cucumber Mosaic Virus* – *CMV1* 5'-UTR, *CMV2* 5'-UTR
- *Potato Virus X* – *PVX* 5'-UTR
- *Tobacco Mosaic Virus* – TMV 5'-UTR
- *African Cassava Mosaic Virus* – RNAi ds*AC1* targeting *Replication Associated Gene AC1*

Chimeric genes and RNA interference:
- *Arabidopsis thaliana* – *Outer envelope protein7* (*OEP7*) translational fusion with *Saccharomyces cerevisiae Hexokinase HxK2* (see below) to anchor the fusion protein to the outer plastid envelope membrane, Chimeric *Rubisco Activase* gene (larger portion of the sequence)
- *Nicotiana tabacum* – *Rubisco Small Subunit41* (*RbcS41*) chloroplast transit peptide, Chimeric *Rubisco Activase* gene (smaller portion of the sequence)
- *African Cassava Mosaic Virus* – RNAi ds*AC1* targeting *Replication Associated Gene AC1*
- *Escherichia coli* – *Glycolate Dehydrogenase* (*GlyDH*) fusion of subunits DEF
- *Saccharomyces cerevisiae* – *Hexokinase2* (*HxK2*) coding sequence fused to *OEP7* (see above)

All coding sequences in the transgenic plants encode proteins functional in plant primary carbon metabolism (transport proteins or enzymes). Therefore, the source of the transgenic material will not affect the safe conduct of a confined field trial.
Please refer to Annexes B for further details regarding the genetic elements within the constructs used for transformation of the cassava clone to be used for this CFT.

Changes in Toxicity or Plant Composition:
[Describe any changes or toxicity, allergenicity, or significant changes in composition intended by the genetic modification.]

There are no expected changes in toxicity or allergenicity of transgenic cassava lines. The transgenic cassava will have higher starch yield on dry weight basis compared with the wild type i.e. non-transgenic.

Describe the Features of the Genetic Construct:
[Include coding sequences, promoters, enhancers, termination and polyadenylation signal sequences. Attach a genetic map and describe the method of modification in an annex.]

The following three transformation plasmids have been used for plant transformation. Description of vectors and genetic elements used, expected attributes of transgenic plants, method of modification, genetic map, coding sequences, and literature cited are illustrated in the respective Annexes B.

Transformation plasmid 1: *p134GG_GPT – NTT – TMT – RNAi AC1* (for details see Annex B1)

Transformation plasmid 2: *p134GG_GPT – NTT – TMT – GlyDH – RNAi AC1* (for details see Annex B2)

Transformation plasmid 3: *p134GG_GPT – NTT – OEP7-HxK – GlyDH – TMT – RCase – RNAi AC1* (for details see Annex B3)

The same selectable marker cassette was used for all plasmids. It includes *Hygromycin Phosphotransferase2* (*Hpt2*) gene that confers resistance to the antibiotic hygromycin B, under the control of the *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*) and the *CaMV35S* terminator. The vector backbone p134GG, used for all plasmid constructions, has been described in detail in Annex B4.

3. Trial Description

This section describes the purpose of the field trial, the experimental design and data to be collected, including anticipated pesticides use. Include a description of the habitat at the site, and any organisms of conservation concern that may be in the general area.

Trial Description:

The purpose of the field trial is to evaluate the effect of the combinatorial over-expression and/or downregulation of genes with potential impact on source and sink capacities, on storage root starch yield in transgenic cassava.
The field trial will be conducted within a screen-house located within the confined field trial site, (see annex A) to reduce the risk of infestation of *Bemisia tabaci*, the whitefly vectoring cassava mosaic disease (CMD), to which the parental cv60444 cultivar is susceptible. Plant leaves will be weekly sprayed, alternating (every 4 weeks) insecticide between *Imidacloprid* and *Lambda-cyhalothrin* (active ingredients).
The experiments will be conducted in 3 screen-houses. In total, up to 30 lines with up to 9 plants per line, plus 30 control (wildtype) plants will be grown, i.e. up to 270 transgenic plants and 30 wildtype plants.

Data will be collected 2 months after planting, 4 months after planting, and at harvest 12 months after planting. Plants will be scored for growth parameters (plant height, number of leaves and nodes, number and root size) and CMD infection.

Source leaves, sink leaves, storage roots and stems will be sampled during data collection in order to determine starch content, metabolites content, and gene expression.

The trial site is in the forest-savannah transition agro-ecology of Nigeria (see Annex C). The site was cleared of the native forest several decades ago. Therefore, there are no known organisms of conservation concern at the test site. Pockets of the original forest that exist in the area are well separated from the test site.

4. Genetic Confinement

This section describes the measures to be taken to ensure confinement of the genetically modified plants and genes. It is based on knowledge of the unmodified crop and the intended genetic modification.

Provide a map showing the location of the trial site, surrounding fields and relevant geographic features such as streams or waterways.

The trial site is located at the IITA headquarters station in Ibadan, capital of Oyo State in South-Western Nigeria. An isolation distance of 100m will be maintained between the CFT site and any plants capable of hybridizing with cassava. See Annex A for an aerial image of the confined field trial site and Annex C for an aerial image of the location of the trial site, surrounding fields and other geographical features.

Are there wild plant species in the vicinity of the trial site that could be fertilized by pollen from the trial plants, resulting to viable seeds?

There are no plants of wild species near the confined field that could be fertilized by cassava pollen. The only wild *Manihot* species in Nigeria is *Manihot glaziovii* (Bock, 1984) which is a non-indigenous ornamental tree species without weedy characteristics. Only few *Manihot glaziovii* plants are kept in the IITA collection within the IITA campus. However, they are located more than 1000m away from the confined field site and are therefore not at risk to be pollinated by trial plants.

Describe mechanisms in place to prevent pollen-mediated gene flow from the plants in the trial site:

[Genetic confinement or reproductive isolation measures are based on the biology of the unmodified plant and the introduced genetic modification, and include isolation distance and /or other measures as justified by the reproductive biology of the unmodified plants, and any intended effects of the introduced traits on their reproductive biology.]

An isolation distance of at least 100m will be maintained between the confined field site and any other cassava field in accordance with the standard for separation used in cassava breeding programs (Kawano et al., 1978). This isolation distance is calculated starting from the outermost border row. The isolation zone will be regularly monitored during and after the trial to ensure the continued absence of any cassava.

Pollen dispersal will be prevented by removal of any male or female flowers during the entire duration of the experiment. In cassava, inflorescence production is preceded by three-way branching of the main stem, making it easy to detect early stages of the flowering process. Weekly monitoring for initiation of inflorescences will take place starting from 2 months after planting. Any inflorescence will be removed and destroyed before maturation. Therefore, there will be no possibility for pollen production or distribution.

There are no effects of the introduced traits intended or expected to impact reproductive biology of the test plants.

Describe measures in place to control trial plant volunteers after termination of the trial:

[Describe the crops to be allowed following the confined trial, duration of monitoring or volunteers, frequency of monitoring, methods of destruction and disposal of any identified volunteers, and any other measures needed to ensure that the trial plants do not persist on the trial site.]

The trial spot area will be left fallow and kept free of volunteer cassava plants for at least one year after the conclusion of the trial or until a new trial will be started. The presence of volunteers will be monitored on a monthly basis for a period of six months after end of trial, and volunteer plants found will be uprooted, left to air dry, and incinerated.

5. Material Confinement

This section describes the mechanisms by which trial personnel will maintain control of the genetically modified plant material, so that it is not mixed with non-modified plant material, does not escape into the environment, and is not eaten by humans or livestock.

Packaging:

[Describe how the genetically modified plant material will be packaged and labeled for transport to the trial site and measures for cleaning and/or disposing of the packaging material. Note that the chain of custody documentation is required for all genetically modified material being transported.]

The experimental plants will be imported from ETH Zurich, Switzerland. Individual plants will be transported in 50ml clear plastic sealed culture tubes each labeled with an appropriate identifier unique to the respective GMO. The tubes will be encased in Styrofoam packaging enclosed in plastic bags and placed in carton boxes. These boxes will be shipped by express courier from Switzerland to IITA headquarters, Ibadan, where they will be received by the IITA principal investigator and transported to the biosafety level 2 (BL2) containment facility. After post-entry inspection and clearance by the regulators, plants will be acclimatized and hardened for 6-8 weeks in sterile soil in individual 15cm pots. After hardening, plants will be packaged in closed cartons or wooden boxes lined with plastic film to prevent the spillage of any material. These cartons will be transported within enclosed vehicles from the screen house to the fenced field trial site within IITA headquarters under the supervision of a representative of IITA Institutional Biosafety Committee (IBC) and the IITA principal investigator.
The packaging, including the plastic tubes, the Styrofoam boxes, plastic bags, cartons, pots, will be destroyed by burning with a flammable liquid in the incinerator within the CFT site.
A compliance form, material transfer form, and import permit will be prepared and maintained to document the movement of plants to the IITA headquarters containment facility from Zurich, Switzerland, and with IITA from the containment facility to the confined field site.

Harvesting, Transport and Storage:

[Describe how the plant material will be harvested, including plans for any material to be retained, and how that material will be stored and/or transported.]

Destructive sample collection and harvesting will be performed in the CFT site within screen houses. Samples will be snap-frozen in liquid nitrogen or stored in sealed plastic bags and transported to the IITA Ibadan BL2 laboratory on campus for further analyses, using established protocols.

Disposal and Clean-up:

[Describe how surplus planting material will be disposed of at the trial site, how any equipment used during plating or other farm operations will be cleaned, and how harvested materials and crop residues will be disposed.]

Surplus planting material will be retained within the BL2 containment facility at IITA headquarters, Ibadan. These plants will be a source of replacement of plants in case of failure of plant establishment

in the confined field site thereby ensuring proper field experimentation.
All plant material will be harvested by hand. Once plant sampling is completed and all data collected, plants will be dug out, chopped up and allowed to air dry for 2-3 days before they will be destroyed by incineration within the fenced CFT site at IITA. Disposal of all material will be recorded in the compliance binder. All tools used for taking samples or harvest will be washed and cleaned and stored at the trial site.

Site Security:
[Describe measures in place to ensure security of the trial site to prevent incursion by humans or animals. Measures may include fencing, security patrols, lockable gates, etc…]

The confined field trial (CFT) site is within the IITA campus in Ibadan that is fenced along the entire perimeter and has restricted access to only authorized personnel.
The CFT site is surrounded by a two meters high chain-linked fencing buried in soil, and has a locked gate to prevent any unauthorized access by people or animals (see Annex A). Moreover, as transgenic plants will be grown in screen houses included in the CFT site, their risk of getting in contact with birds is extremely low.
A guardhouse is also existing at the gate to allow for 24 hour security (see Annex A). To ensure that material confinement standards are met, only approved personnel will have access to the area. A logbook will be maintained to keep records of all visitors to the site, and access will be allowed only to authorized personnel.

6. Records, Personnel, and Planning

Records and Documentation:
[Describe measures in place to ensure adequate documentation of all confinement measures and data requirements as described herein.]

All completed material transfer agreement forms will be incorporated into the compliance binder maintained at the CFT site and will be available at all times for review by Nigerian biosafety inspectors and regulators.
The **compliance forms** to be completed and maintained will include:
Material transfer forms to document movement of the plants to IITA containment facility from ETH Zurich, Switzerland, and to IITA CFT site from the IITA containment facility.
Confined field trial form to document the exact number of transgenic cassava plants of each modified line planted in soil in the CFT site, the exact number and purpose of any plants not planted but retained in the containment facility as well as the destruction of shipping containers.
Weekly flower bud removal form to document the weekly inspection for scoring and removal of flower buds. An additional document will be created to document flower bud destruction in the incineration pit.
Monthly isolation monitoring form to document the monthly inspection of the 100m isolation distance as well as to document the destruction of any prohibited plants found to be within the isolation area.
Fertilizer, pesticide and insecticide usage form to document fertilizer, pesticide and insecticide application during and after the CFT.
Incident and corrective action form. In case of any breach of confinement, IITA IBC and National Biosafety office will be immediately notified of the incident by phone, and the corrective action form will be completed.
Isolation distance monitoring form, to document the weekly inspection of the isolation distance and ensure that no cassava plant is established within this area.

Harvest and destruction form to document the harvest and destruction of all cassava plants within the CFT site.
Post-harvesting volunteer monitoring form to document the monthly post-harvest inspection of the CFT site for cassava plants and destruction of these volunteers.
Transfer to BL2 containment facility and laboratory form to document the transfer of harvested plant material to the containment facility and laboratory for further analysis.
The **data forms** to be completed and maintained will include:
Records of plants in the containment facility form to document and monitor number and general health of plantlets during hardening.
CMD infection evaluation form to document occurrence of CMD infection and its severity (1-5 scale scoring) on each individual plant on a bi-weekly basis for the entire duration of the trial.
Monthly plot observation form to record data on plant height, occurrence and severity of cassava bacterial blight, anthracnose, infestation by green mites, whiteflies, mealybugs and any general comments.
Meteorological data form to report rainfall, relative humidity and temperature recorded daily by the weather station of IITA Ibadan.

Personnel:
[Describe measures in place to ensure that trial personnel will have appropriate education, experience and training to adequately perform assigned duties for confinement and technical requirements of the trial.]

Trial personnel have skills in biotechnology and will be appropriately trained in biosafety issues to cope with the requirements of this study. The principal investigator has long standing experience in biotechnology and compliance with biosafety regulations. The CFT manager has documented experience in biotechnology and in confined field and screen-house management. A list of authorized personnel for the different activities related to the CFT will be prepared and stored in the compliance binder at the CFT site.

Contingency Plans:
[Describe planned response to the loss of control or accidental release of genetically modified plant material, including notification of authorities and the Authorized Party, recovery and disposal of plant material, and any other measures to be taken to mitigate any potential adverse effects.]

In the highly unlikely event of an accidental release of genetically modified plant material, the IITA IBC and Nigerian Biosafety officials will be notified immediately and will in addition receive a written notification within 24 hours to become aware of the incident. An incident and corrective action form will be completed for any case of an accidental release. The completed form will be incorporated in the compliance binder and maintained at the CFT site.
In the unlikely event that transgenic plantlets fall out of their sealed tubes, packaging materials and carrying bag during transport, the plantlets will be immediately recovered and returned to their storage tube with subsequent destruction by incineration. Nigerian Biosafety officials will be notified immediately of the occurrence. If any plantlet spills from its pot in the closed carton during transport, it will be similarly recovered and subsequently destroyed.
If any plants are accidentally removed from the trial site after planting, biosafety regulators will be notified immediately of the event, and efforts will be undertaken to recover the material under the guidance of the biosafety desk office.
The biosafety inspectors will also be notified immediately of any unintended violation of reproductive

isolation. If the breach in the reproductive isolation would be due to a cassava plant flowering inside the CFT site, the 100m isolation distance will ensure that genetic confinement is maintained.
In the unlikely event of unmanageable civil unrest or a natural disaster that affects the integrity of the CFT site, biosafety regulators will be notified and the entire experimental material will be destroyed.

7. Declaration

I hereby certify that the information in the application and all attachments is complete and accurate to the best of my knowledge and belief:

Signature of Principal Investigator for Applying Institution:

Date:

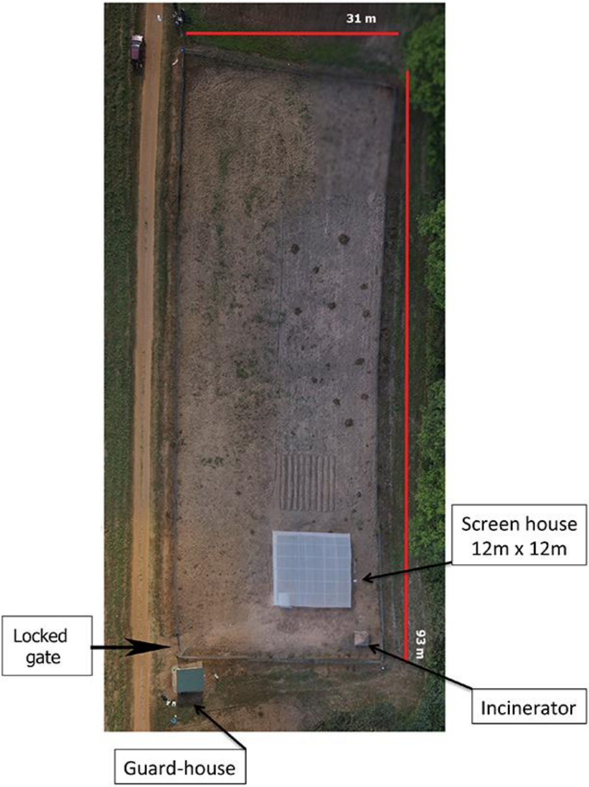
Signature of Lead Scientist of Collaborating Institution:

Date:

ANNEX A

Aerial picture of the site of the confined field trial. Dimensions and critical elements are indicated in the picture.

IITA confined field aerial picture



Annex B1

GPT – *NTT* – *TMT* – D4 – D5 – D6 – RNAi *AC1*

<u>Content</u>

I. <u>Description of vectors and genetic elements used for plant transformation</u>

(a) Transformation plasmid

p134GG_GPT – NTT – TMT – D4 – D5 – D6 – RNAi AC1

(b) Functional cassettes

<u>GPT</u>

Promoter – *Manihot esculenta* (Cassava) *Granule Bound Starch Synthase1* (*MeGBSS1*; Koehorst van Putten *et al.*, 2012)
5′-UTR-CDS – *Pisum sativum Glucose 6-phosphate/Phosphate Translocator* (*PsGPT*; Kammerer *et al.*, 1998)
3′-UTR-terminator – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*) terminator

<u>NTT</u>

Promoter – *Solanum tuberosum Soluble Starch Synthase3* (*StSSS3*)
5′-UTR – *CMV1* from *Cucumber Mosaic Virus*
CDS – *Arabidopsis thaliana Nucleoside Triphosphate Translocator1* (*AtNTT1*; Tjaden *et al.*, 1998)
3′-UTR-terminator – *Agrobacterium tumefaciens Mannopine Synthase* (*AtuMAS*) terminator

<u>TMT</u>

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit3B* (*AtRbcS3B*)
5′-UTR – *PVX* from *Potato Virus X*
CDS – *Arabidopsis thaliana Tonoplast Monosaccharide Transporter1* (*AtTMT1*; Wingenter *et al.*, 2010)
3′-UTR-terminator – *Agrobacterium tumefaciens* Gene7 (*AtuG7*) terminator

<u>D4, D5, D6</u>

Empty cloning site (D) 4, 5, 6

<u>RNAi *AC1*</u>

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit2B* (*AtRbcS2B*)
CDS – *RNAi dsAC1* targeting the *Replication Associated Gene AC1* of *African Cassava Mosaic Virus* (*ACMV)*
3'-UTR-terminator – *Cauliflower Mosaic Virus Gene 35S* (*CaMV35S*) terminator

(c) Selectable marker cassette

Promoter – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*)
CDS – *Hygromycin Phosphotransferase2* (*Hpt2*)
Terminator – *CaMV35S*

II. <u>Expected outcome of transgenic plants</u>

(a) Combined overexpression of *PsGPT* and *AtNTT1* in Cassava storage roots

Cassava converts assimilates produced by photosynthesis into root starch for long-term storage. Root starch is stored in the amyloplasts of storage roots. Starch synthesis in the amyloplasts ultimately depends on the import of energy in the form of adenosine triphosphate (ATP) and carbon building blocks in the form of glucose-6-phosphate. Glucose-6-phosphate is taken up into amyloplasts by a membrane-localized transporter called *Glucose 6-phosphate/Phosphate Translocator* (Kammerer *et al*., 1998). ATP is imported into amyloplasts via the *Nucleoside Triphosphate Translocator1* (Tjaden *et al*., 1998). Simultaneous overexpression of *PsGPT* and *AtNTT1* in the storage roots should result in an increased uptake of energy and carbon building blocks into amyloplasts and should therefore result in an increased root starch content. Previously, this concept was demonstrated by overexpression experiments using transgenic potato plants (Jonik et al., 2002, Zhang et al., 2008). It is expected, that Cassava plants overexpressing both *PsGPT* and *AtNTT* will display an increased starch content in the storage roots.

(b) Overexpression of *AtTMT1* in Cassava leaves

AtTMT1 encodes a membrane protein, responsible for transferring glucose from the plant cytosol into the vacuolar compartment. In the model plant *Arabidopsis thaliana*, it has been shown that overexpression of *TMT1* results in an increased seed yield. Overexpression of this protein led to an increased expression of photosynthesis-related genes, reduced consumption of sugars for cellular respiration, reduced nocturnal loss of CO_2, and increased sugar export capacity from source leaves (Wingenter *et al*., 2010). It is expected, that Cassava plants overexpressing *AtTMT1* show an overall increase photosynthetic carbon fixation and export to the roots, thereby increasing final root yield.

(c) RNA Interference targeting *ACMV AC1*

ACMV causes significant economic losses for Cassava production. Previous experiments demonstrated that expressing *ACMV AC1*-homologous hairpin double-strand RNAs increases the resistance of Cassava against *ACMV* (Vanderschuren *et al*., 2009). It is expected that Cassava plants expressing the RNAi construct targeting the *ACMV AC1* will display an increased resistance against the pathogen.

III. Method of modification

Genetic modification of the cassava cultivar 60444 has been done following the method described by Bull *et al.* (2009). In brief, friable embryogenic calli (FEC) have been transformed with *Agrobacterium tumefaciens* containing the binary vector *p134GG_GPT – NTT – TMT – D4 – D5 – D6 – RNAi-AC1* described above. Hygromycin-resistant embryos have been regenerated and screened to confirm the presence of the transgene.

IV. Genetic map

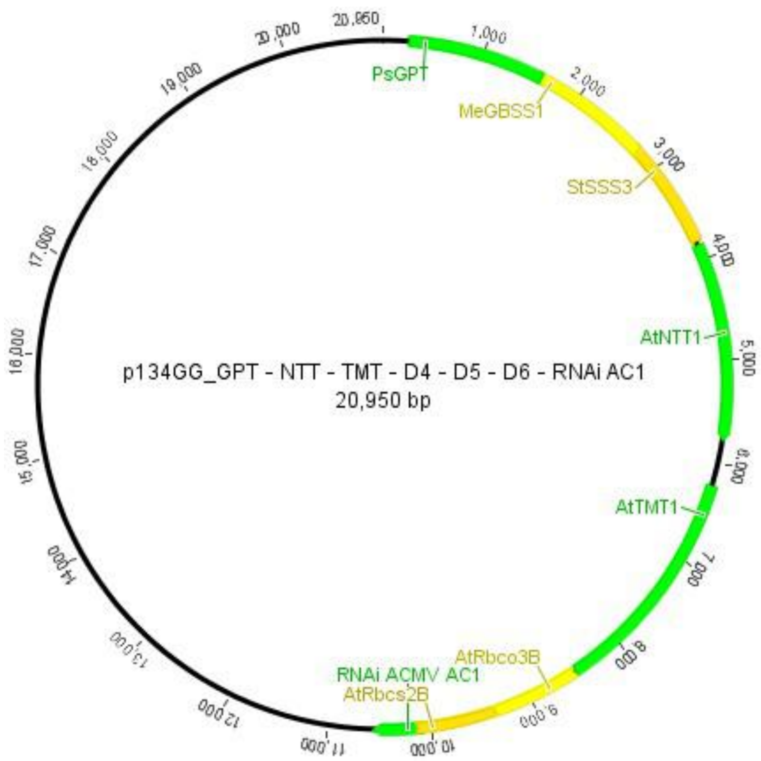


Fig. 1: Vector map of transformation plasmid *p134GG_GPT – NTT – TMT – D4 – D5 – D6 – RNAi AC1*

V. Sequence (from left border to right border)

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*TGTGACCGA***GGGA**ATCGGGAATTAAACTATCAGTGTTTGACAGGATATATTGGCGGGTAAAC

Legend:

LB – *CaMV35S* 3′-UTR and terminator – *HPTII* coding sequence – *AtuNOS* promoter – *AtuNOS* 3′-UTR and terminator – *PsGPT* 5′-UTR and coding sequence – *MeGBSS1* promoter – *StSSS3* promoter – *CMV1* 5′-UTR – *AtNTT1* coding sequence – *AtuMAS* 3′-UTR and terminator – *AtuG7* 3′-UTR and terminator – *AtTMT1* coding sequence – *PVX* 5′-UTR – *AtRbcS3B* promoter – D4 – D5 – D6 – *AtRbcS2B* promoter – 5′-UTR link – RNAi ds*AC1* sequence – *CaMV35S* 3′-UTR and terminator – RB

VI. Expression of the transcriptional units

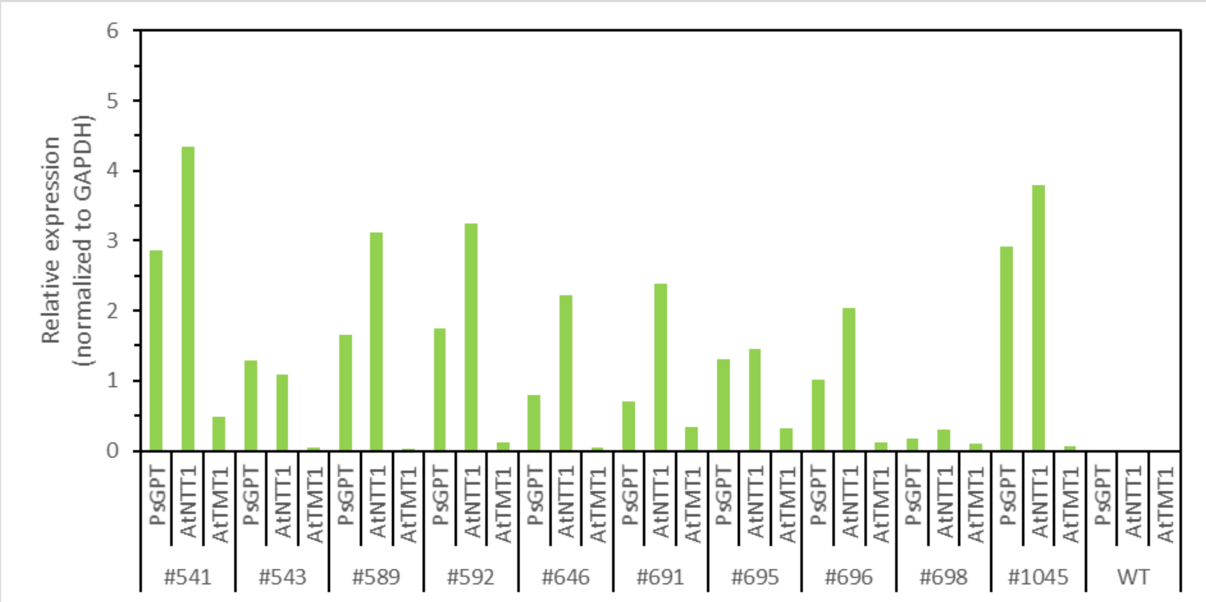

Fig. 2: Relative expression of *PsGPT*, *AtNTT1* and *AtTMT1* in leaves of the lines #541, 543, 589, 592, 646, 691, 695, 696, 698 and 1045. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

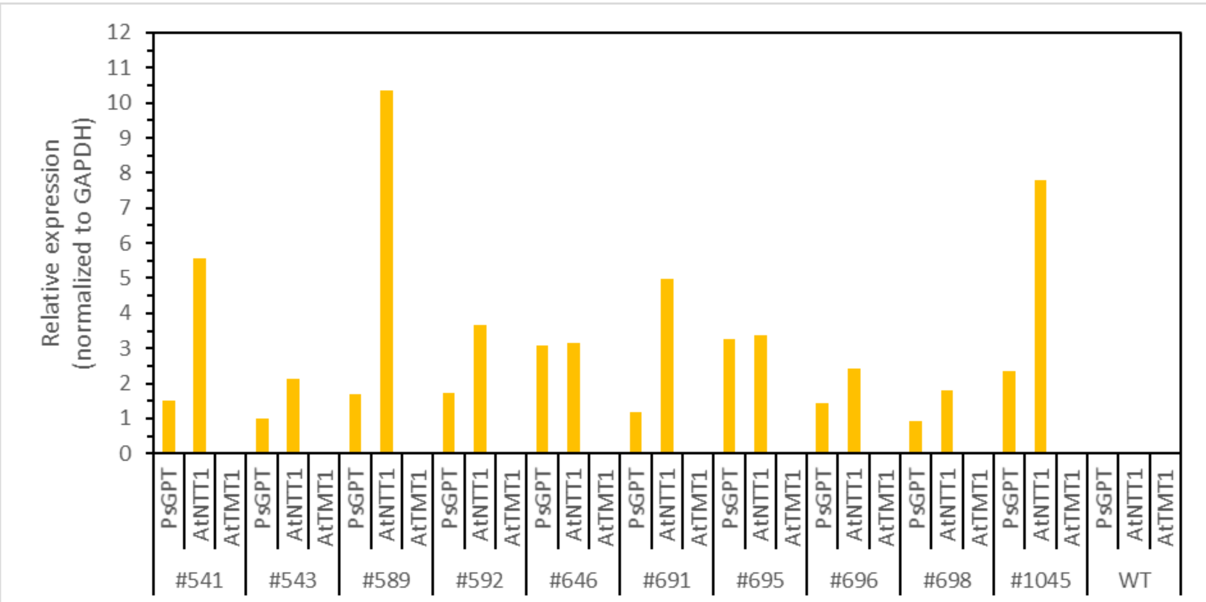

Fig. 3: Relative expression of *PsGPT*, *AtNTT1* and *AtTMT1* in roots of the lines #541, 543, 589, 592, 646, 691, 695, 696, 698 and 1045. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

VII. <u>Literature cited</u>

Bull SE, Owiti JA, Niklaus M, Beeching JR, Gruissem W, Vanderschuren H (2009). Agrobacterium-mediated transformation of friable embryogenic calli and regeneration of transgenic cassava. *Nat Protoc* 4:1845-54.

Jonik C, Sonnewald U, Hajirezaei MR, Flügge UI, Ludewig F (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnol J* 10(9):1088-98

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge U-I (1998). Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose-6-phosphate/phosphate antiporter. *Plant Cell* 10:105-17.

Koehorst-van Putten HJJ, Wolters A-MA, Pereira-Bertram IM, van den Berg HHJ, van der Krol AR, Visser RGF (2012). Cloning and characterization of a tuberous root-specific promoter from cassava (*Manihot esculenta* Crantz). *Planta* 236:1955-65.

Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998). Altered plastidic ATP/ADP-translocator activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant J* 16, 531-40.

Vanderschuren H, Alder A, Zhang P, Gruissem W (2009). Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop Cassava. *Plant Mol Biol.* 70(3):265-72

Wingenter K, Schulz A, Wormit A, Wic S, Trentmann O, Hoermiller II, Heyer AG, Marten I, Hedrich R, Neuhaus HE (2010). Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in Arabidopsis. *Plant Physiol* 154, 665-77.

Zhang L, Haeusler RE, Greiten C, Hajirezaei MR, Haferkamp I, Neuhaus HE, Flügge UI, Ludewig F (2008). Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol J* 6(5):453-64

Annex B2

GPT – *NTT* – *TMT* – *GlyDH* – D5 – D6 – RNAi *AC1*

Content

I. Description of vectors and genetic elements used for plant transformation

(a) Transformation plasmid

p134GG_GPT – NTT – TMT – GlyDH – D5 – D6 – RNAi AC1

(b) Functional cassettes

GPT

Promoter – *Manihot esculenta Granule Bound Starch Synthase1* (*MeGBSS1*; Koehorst van Putten *et al.*, 2012)
5'-UTR-CDS – *Pisum sativum Glucose 6-phosphate/Phosphate Translocator* (*PsGPT*; Kammerer *et al.*, 1998)
3'-UTR-terminator – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*) terminator

NTT

Promoter – *Solanum tuberosum Soluble Starch Synthase3* (*StSSS3*)
5'-UTR – *CMV1* from *Cucumber Mosaic Virus*
CDS – *Arabidopsis thaliana Nucleoside Triphosphate Translocator1* (*AtNTT1*; Tjaden *et al.*, 1998)
3'-UTR-terminator – *Agrobacterium tumefaciens Mannopine Synthase* (*AtuMAS*) terminator

TMT

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit3B* (*AtRbcS3B*)
5'-UTR – *PVX* from *Potato Virus X*
CDS – *Arabidopsis thaliana Tonoplast Monosaccharide Transporter1* (*AtTMT1*; Wingenter *et al.*, 2010)
3'-UTR-terminator – *Agrobacterium tumefaciens Gene7* (*AtuG7*) terminator

GlyDH

Promoter – *Solanum tuberosum leaf specific1* (*StLS1*)
5'-UTR – *TMV* from *Tobacco Mosaic Virus*

Chloroplast transit peptide – from *RbcS41* of *Nicotiana tabacum*
CDS – *E. coli Glycolate dehydrogenase* (*EcGlyDH*) fusion of subunits DEF
3'-UTR-terminator – *Agrobacterium tumefaciens Octopine synthase* (*AtuOCS*) terminator

D5, D6

Empty cloning site (D) 4, 5, 6

RNAi *AC1*

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit2B* (*AtRbcS2B*)
CDS – RNAi ds*AC1* targeting the *Replication Associated Gene AC1* of *African Cassava Mosaic Virus* (ACMV)
3'-UTR-terminator – *Cauliflower Mosaic Virus Gene 35S* (*35S*) terminator

(c) Selectable marker cassette

Promoter – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*)
CDS – *Hygromycin Phosphotransferase2* (*Hpt2*)
Terminator – *CaMV35S*

II. Expected outcome of transgenic plants

(a) Combined overexpression of *PsGPT* and *AtNTT1* in Cassava storage roots

Cassava converts assimilates produced by photosynthesis into root starch for long-term storage. Root starch is stored in the amyloplasts of storage roots. Starch synthesis in the amyloplasts ultimately depends on the import of energy in the form of adenosine triphosphate (ATP) and carbon building blocks in the form of glucose-6-phosphate. Glucose-6-phosphate is taken up into amyloplasts by a membrane-localized transporter called *Glucose 6-phosphate/Phosphate Translocator* (Kammerer *et al.*, 1998). ATP is imported into amyloplasts via the *Nucleoside Triphosphate Translocator1* (Tjaden *et al.*, 1998). Simultaneous overexpression of *PsGPT* and *AtNTT1* in the storage roots should result in an increased uptake of energy and carbon building blocks into amyloplasts and should therefore result in an increased root starch content. Previously, this concept was demonstrated by overexpression experiments using transgenic potato plants (Jonik et al., 2002, Zhang et al., 2008). It is expected, that Cassava plants overexpressing both *PsGPT* and *AtNTT* will display an increased starch content in the storage roots.

(b) Overexpression of *AtTMT1* in Cassava leaves

AtTMT1 encodes a membrane protein, responsible for transferring glucose from the plant cytosol into the vacuolar compartment. In the model plant *Arabidopsis thaliana*, it has been shown that overexpression of *TMT1* results in an increased seed yield. Overexpression of this protein led to an increased expression of photosynthesis-related genes, reduced consumption of sugars for cellular respiration, reduced nocturnal loss of CO_2, and increased sugar export capacity from source leaves (Wingenter *et al*., 2010). It is expected, that Cassava plants overexpressing *AtTMT1* show an overall increase photosynthetic carbon fixation and export to the roots, thereby increasing final root yield.

(c) Overexpression of *EcGlyDH* in Cassava leaves

High temperatures increase the likelihood of *Ribulose-1,5-bisphosphate-caboxylase/-oxygenase* (*Rubisco*) to bind O_2 instead of CO_2. While the binding of CO_2 results in the normal photosynthetic carbon fixation via the generation of two molecules 3-phosphoglycerate in the Calvin Benson Cycle, the binding of O_2 creates the toxic reaction product 2-phosphoglycolate. The regeneration of 2-phosphoglycolate back to 3-phosphoglycerate, a process called photorespiration, happens in three different plant compartments and is one of the most inefficient and costly reactions present in plant cells. Expression of the EcGlyDH polyprotein in the plastids of plant cells has been demonstrated to function as a photorespiratory bypass by converting 2-phosphoglycolate to glycerate and CO_2 directly in the plastids. This photorespiratory bypass was reported to increase both the yield of *Camelina sativa* (Dalal *et al*., 2015) and *Solanum tuberosum* (Nölke *et al.*, 2014). In potato, overexpression of *EcGlyDH* resulted in increased photosynthesis, significantly higher sugar and starch levels and an approximately two-fold increase in tuber yield under greenhouse conditions. It is expected that overexpression of *EcGlyDH* in the leaves of Cassava will result in reduced photorespiratory energy loss, increased photosynthetic carbon fixation and higher root starch yield.

(d) RNA Interference targeting *ACMV AC1*

ACMV causes significant economic losses for Cassava production. Previous experiments demonstrated that expressing *ACMV AC1*-homologous hairpin double-strand RNAs increases the resistance of Cassava against *ACMV* (Vanderschuren *et al*., 2009). It is expected that Cassava plants expressing the RNAi construct targeting the *ACMV AC1* will display an increased resistance against the pathogen.

III. Method of modification

Genetic modification of the cassava cultivar 60444 has been done following the method described by Bull et al. (2009). In brief, friable embryogenic calli (FEC) have been transformed with *Agrobacterium tumefaciens* containing the binary vector *p134GG_GPT – NTT – TMT – GlyDH – D5 – D6 – RNAi AC1* described above. Hygromycin-resistant embryos have been regenerated and screened to confirm the presence of the transgene.

IV. Genetic map

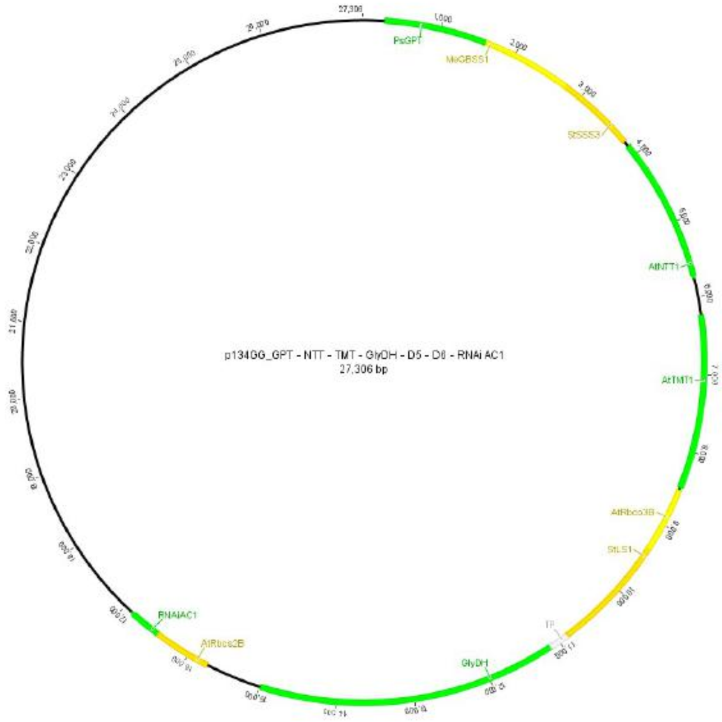

Fig. 1: Vector map of transformation plasmid *p134GG_GPT – NTT – TMT – GlyDH – D5 – D6 – RNAi AC1*

V. Sequence (from left border to right border)

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Legend:

LB – *CaMV35S* 3′-UTR and terminator – *HPTII* coding sequence – *AtuNOS* promoter – *AtuNOS* 3′-UTR and terminator – *PsGPT* 5′-UTR and coding sequence – *MeGBSS1* promoter – *StSSS3* promoter – *CMV1* 5′-UTR – *AtNTT1* coding sequence – *AtuMAS* 3′-UTR and terminator – *AtuG7* 3′-UTR and terminator – *AtTMT1* coding sequence – *PVX* 5′-UTR – *AtRbcS3B* promoter – *StLS1* promoter – 5′-UTR-*TMV-NtRbcS41* – *EcGlyDH* coding sequence – *AtuOCS* 3′-UTR and terminator – D5 – D6 – *AtRbcS2B* promoter – 5′-UTR link – RNAi ds*AC1* sequence – *CaMV35S* 3′-UTR and terminator - RB

VI. Expression of the transcriptional units

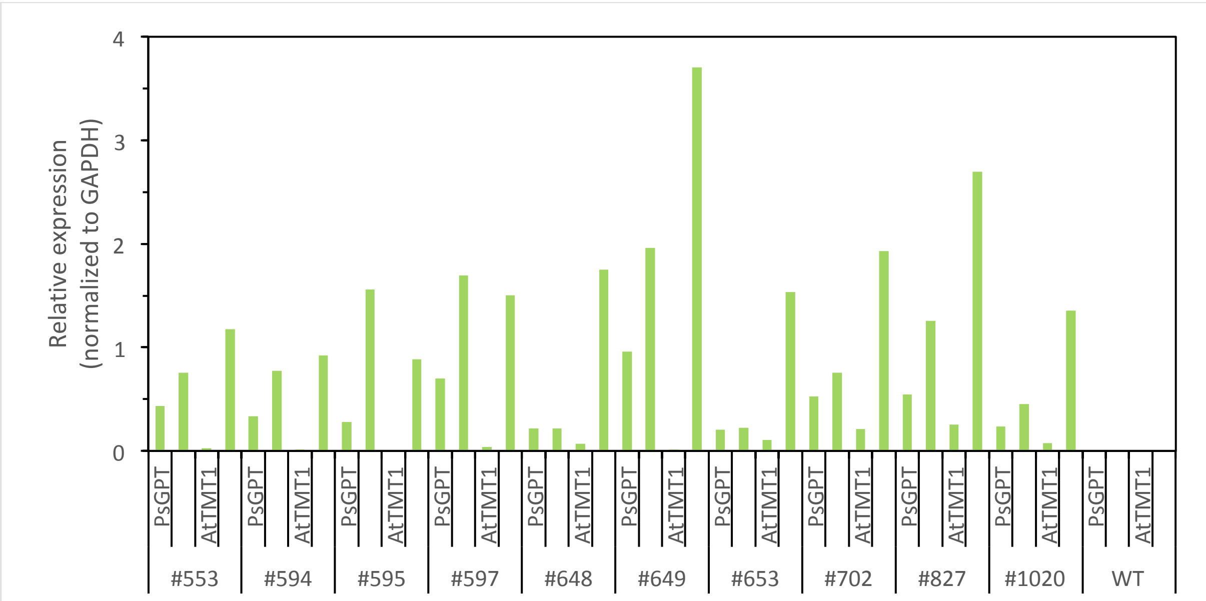

Fig. 2: Relative expression of *PsGPT*, *AtNTT1, AtTMT1* and EcGlyDH in leaves of the lines #553, 594, 595, 597, 648, 649, 653, 702, 827 and 1020. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

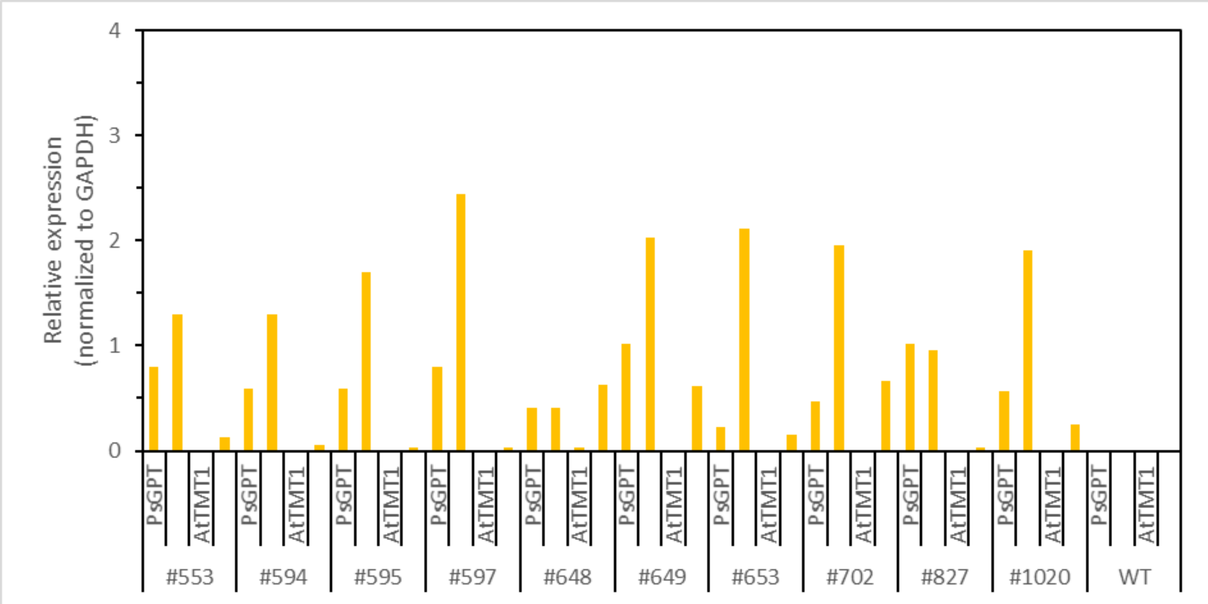

Fig. 3: Relative expression of *PsGPT*, *AtNTT1, AtTMT1* and EcGlyDH in roots of the lines #553, 594, 595, 597, 648, 649, 653, 702, 827 and 1020. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

VII. Literature cited

Bull SE, Owiti JA, Niklaus M, Beeching JR, Gruissem W, Vanderschuren H (2009). Agrobacterium-mediated transformation of friable embryogenic calli and regeneration of transgenic cassava. *Nat Protoc* 4:1845-54.

Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu Rongda, Sederoff HW (2015). A photorespiratory bypass increases plant growth and seed yield in biofuel crop *Camelina sativa*. *Biotechnology for Biofuels* 8:175

Jonik C, Sonnewald U, Hajirezaei MR, Flügge UI, Ludewig F (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnol J* 10(9):1088-98

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge U-I (1998). Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose-6-phosphate/phosphate antiporter. *Plant Cell* 10:105-17.

Koehorst-van Putten HJJ, Wolters A-MA, Pereira-Bertram IM, van den Berg HHJ, van der Krol AR, Visser RGF (2012). Cloning and characterization of a tuberous root-specific promoter from cassava (*Manihot esculenta* Crantz). *Planta* 236:1955-65.

Nölke G, Houdelet M, Kreuzaler F, Peterhänsel C, Schillberg S (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (*Solanum tuberosum*) plastids strongly enhances photosynthesis and tuber yield. *Plant Biotechnol J*. 12(6):734-42

Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998). Altered plastidic ATP/ADP-translocator activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant J* 16:531-40.

Vanderschuren H, Alder A, Zhang P, Gruissem W (2009). Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop Cassava. Plant Mol Biol. 70(3):265-72

Wingenter K, Schulz A, Wormit A, Wic S, Trentmann O, Hoermiller II, Heyer AG, Marten I, Hedrich R, Neuhaus HE (2010). Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in Arabidopsis. *Plant Physiol* 154:665-77.

Zhang L, Haeusler RE, Greiten C, Hajirezaei MR, Haferkamp I, Neuhaus HE, Flügge UI, Ludewig F (2008). Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol J* 6(5):453-64

Annex B3

GPT – NTT – OEP7-HxK – GlyDH – TMT – RCase – RNAi *AC1*

Content

I. Description of vectors and genetic elements used for plant transformation

(a) Transformation plasmid

p134GG_GPT – NTT – OEP7-HxK – GlyDH – TMT – RCase – RNAi AC1

(b) Functional cassettes

GPT

Promoter – *Manihot esculenta Granule Bound Starch Synthase1* (*MeGBSS1*; Koehorst van Putten *et al.*, 2012)
5'-UTR-CDS – *Pisum sativum Glucose 6-phosphate/Phosphate Translocator* (*PsGPT*; Kammerer *et al.*, 1998)
3'-UTR-terminator – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*) terminator

NTT

Promoter – *Solanum tuberosum Soluble Starch Synthase3* (*StSSS3*)
5'-UTR – *CMV1* from *Cucumber Mosaic Virus*
CDS – *Arabidopsis thaliana Nucleoside Triphosphate Translocator1* (*AtNTT1*; Tjaden *et al.*, 1998)
3'-UTR-terminator – *Agrobacterium tumefaciens Mannopine Synthase* (*AtuMAS*) terminator

OEP7-HxK

Promoter – *Solanum tuberosum Starch phosphorylase* (*StSTP*)
5'-UTR-CDS – *Arabidopsis thaliana Outer Envelope Protein7* (*AtOEP7*) fused to *Saccharomyces cerevisiae Hexokinase2* (*ScHxK2*)
3'-UTR-terminator – *Solanum tuberosum H4 terminator*

GlyDH

Promoter – *Solanum tuberosum leaf specific1* (*StLS1*)
5'-UTR – *TMV* from *Tobacco Mosaic Virus*
Chloroplast transit peptide – from *RbcS41* of *Nicotiana tabacum*
CDS – *E. coli Glycolate dehydrogenase* (*EcGlyDH*) fusion of subunits DEF

3′-UTR-terminator – *Agrobacterium tumefaciens Octopine synthase* (*AtuOCS*) terminator

TMT

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit3B* (*AtRbcS3B*)
5′-UTR – *PVX* from *Potato Virus X*
CDS – *Arabidopsis thaliana Tonoplast Monosaccharide Transporter1* (*AtTMT1*; Wingenter *et al.*, 2010)
3′-UTR-terminator – *Agrobacterium tumefaciens Gene7* (*AtuG7*) terminator

RCase

Promoter – *Arabidopsis thaliana Chlorophyll a/b binding protein1* (*AtCAB1*)
5′-UTR – *BSMV* from *Barley Stripe Mosaic Virus*
CDS – *Arabidopsis thaliana* and *Nicotiana tabacum* chimeric *Rubisco activase* (*RCase*)
3′-UTR-terminator – *Solanum lycopersicum ATPase* terminator

RNAi *AC1*

Promoter – *Arabidopsis thaliana Ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit2B* (*AtRbcS2B*)
CDS – RNAi ds*AC1* targeting the *Replication Associated Gene AC1* of *African Cassava Mosaic Virus* (*ACMV*)
3′-UTR-terminator – *Cauliflower Mosaic Virus Gene 35S* (*CaMV35S*) terminator

(c) Selectable marker cassette

Promoter – *Agrobacterium tumefaciens Nopaline Synthase* (*AtuNOS*)
CDS – *Hygromycin Phosphotransferase2* (*Hpt2*)
Terminator – *CaMV35S*

II. Expected outcome of transgenic plants

(a) Combined overexpression of *PsGPT* and *AtNTT1* in Cassava storage roots

Cassava converts assimilates produced by photosynthesis into root starch for long-term storage. Root starch is stored in the amyloplasts of storage roots. Starch synthesis in the amyloplasts ultimately depends on the import of energy in the form of adenosine triphosphate (ATP) and carbon building blocks in the form of glucose-6-phosphate. Glucose-6-phosphate is taken up into amyloplasts by a membrane-localized transporter called *Glucose 6-phosphate/Phosphate Translocator* (Kammerer *et al.*, 1998). ATP is imported into amyloplasts via the *Nucleoside Triphosphate Translocator1* (Tjaden *et al.*, 1998). Simultaneous overexpression of *PsGPT* and *AtNTT1* in the storage roots should result in an increased uptake of energy and carbon building blocks into amyloplasts and should therefore result in an increased root starch content. Previously, this concept was demonstrated by overexpression experiments using transgenic potato plants (Jonik et al., 2002, Zhang et al., 2008). It is expected, that Cassava plants overexpressing both *PsGPT* and *AtNTT* will display an increased starch content in the storage roots.

(b) Overexpression of the *Arabidopsis thaliana Outer Envelope Protein 7* (*AtOEP7*) and *Saccharomyces cerevisiae Hexokinase 2* (*ScHxK2*) fusion protein in Cassava storage roots

Starch synthesis depends on glucose-6-phosphate import into amyloplasts. *Saccharomyces cerevisiae Hexokinase 2* can phosphorylate glucose to glucose-6-phosphate. Anchoring this enzyme to the outer envelope of storage root amyloplasts could lead to an increased synthesis of glucose-6-phosphate in the proximity of the Glucose-6-phosphate/Phosphate Translocator and therefore to increased glucose-6-phosphate uptake into amyloplasts. Increase glucose-6-phosphate uptake into amyloplasts should lead to increased starch synthesis. It is expected that Cassava plants overexpression *AtOEP7-HxK2* have increased levels of storage root starch.

(c) Overexpression of *EcGlyDH* in Cassava leaves

High temperatures increase the likelihood of *Ribulose-1,5-bisphosphate-caboxylase/-oxygenase* (*Rubisco*) to bind O_2 instead of CO_2. While the binding of CO_2 results in the normal photosynthetic carbon fixation via the generation of two molecules 3-phosphoglycerate in the Calvin Benson Cycle, the binding of O_2 creates the toxic reaction product 2-phosphoglycolate. The regeneration of 2-phosphoglycolate back to 3-phosphoglycerate, a process called photorespiration, happens in three different plant compartments and is one of the most inefficient and costly reactions present in plant cells. Expression of the EcGlyDH polyprotein in the plastids of plant cells has been demonstrated to function as a photorespiratory bypass by converting 2-phosphoglycolate to glycerate and CO_2 directly in the plastids. This photorespiratory bypass was reported to increase both the yield of *Camelina sativa* (Dalal *et al*., 2015) and *Solanum tuberosum* (Nölke *et al.*, 2014). In potato, overexpression of *EcGlyDH* resulted in increased photosynthesis, significantly higher sugar and starch levels and an approximately two-fold increase in tuber yield. It is expected that overexpression of *EcGlyDH* in the leaves of Cassava will result in increased photosynthetic carbon fixation and higher root starch yield.

(d) Overexpression of *AtTMT1* in Cassava leaves

AtTMT1 encodes a membrane protein, responsible for transferring glucose from the plant cytosol into the vacuolar compartment. In the model plant *Arabidopsis thaliana*, it has been shown that overexpression of *TMT1* results in an increased yield. Overexpression of this protein led to an increased expression of photosynthesis-related genes, reduced consumption of sugars for cellular respiration, reduced nocturnal loss of CO_2, and increased sugar export capacity from source leaves (Wingenter *et al*., 2010). It is expected, that Cassava plants overexpressing *AtTMT1* show an overall increase photosynthetic carbon fixation and export to the roots, thereby increasing final root yield.

(e) Overexpression of chimeric *Arabidopsis thaliana* and *Nicotiana tabacum Rubisco activase* (*RCase*) in Cassava leaves

Photosynthetic activity decreases with increasing temperature. Even a tropical plant like Cassava loses photosynthetic efficiency at temperatures above 35°C. As temperature increases, the rate of Rubisco deactivation exceeds the capacity of the RCase to promote activation (Crafts-Brandner *et al.*, 2000). This can result in decreased photosynthesis and Cassava root yield at very high temperatures. Previous experiments in *Arabidopsis thaliana* demonstrated that overexpression of a thermostable chimeric RCase leads to better growth and higher rates of photosynthesis at high temperatures (Kumar *et al.*, 2009). It is expected that overexpression of this thermostable chimeric RCase increases the photosynthesis in Cassava leaves and ultimately leads to higher storage root yield.

(f) RNA Interference targeting *ACMV AC1*

ACMV causes significant economic losses for Cassava production. Previous experiments demonstrated that expressing *ACMV AC1*-homologous hairpin double-strand RNAs increases the resistance of Cassava against *ACMV* (Vanderschuren *et al.*, 2009). It is expected that Cassava plants expressing the RNAi construct targeting the *ACMV AC1* will display an increased resistance against the pathogen.

III. Method of modification

Genetic modification of the cassava cultivar 60444 has been done following the method described by Bull *et al.* (2009). In brief, friable embryogenic calli (FEC) have been transformed with *Agrobacterium tumefaciens* containing the binary vector *p134GG_GPT – NTT – OEP7-HxK – GlyDH – TMT – RCase – RNAi AC1* described above. Hygromycin-resistant embryos have been regenerated and screened to confirm the presence of the transgene.

IV. Genetic map

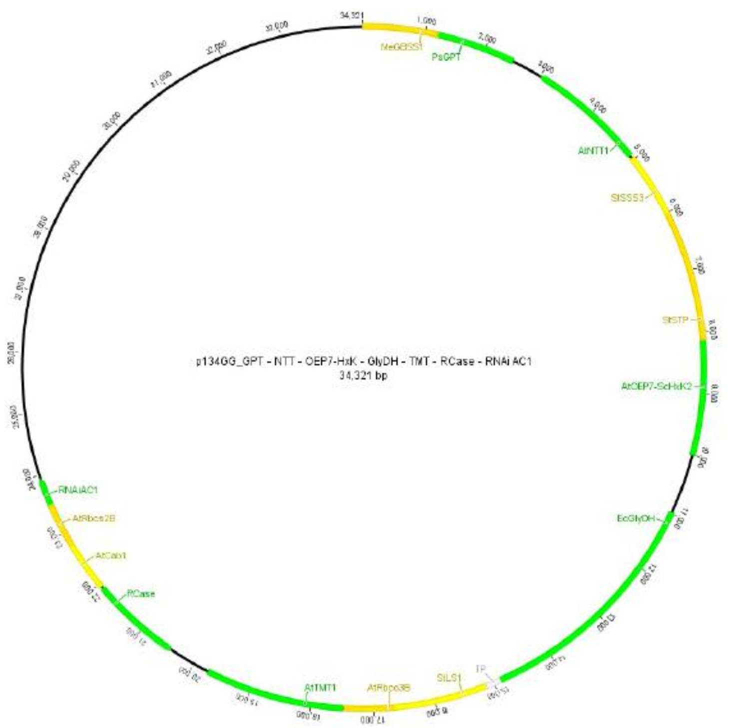

Fig. 1: Vector map of transformation plasmid *p134GG_GPT – NTT – OEP7-HxK – GlyDH – TMT – RCase – RNAi AC1*

V. Sequence (from left border to right border)

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Legend:

LB – *CaMV35S* 3′-UTR and terminator – *HPTII* coding sequence – *AtuNOS* promoter – *MeGBSS1* promoter – *PsGPT* 5′-UTR and coding sequence – *AtuNOS* 3′-UTR and terminator – *AtuMAS* 3′-UTR and terminator – *AtNTT1* coding sequence – *CMV1* 5′-UTR – *StSSS3* promoter – *StSTP* promoter – *AtOEP7-HxK* coding sequence – *StHisH4* 3′-UTR and terminator – *AtuOCS* 3′-UTR and terminator – *EcGlyDH* coding sequence – 5′-UTR-*TMV-NtRbcS41* – *StLS1* promoter – *AtRbcS3B* promoter – *PVX* 5′-UTR – *AtTMT1* coding sequence – *AtuG7* 3′-UTR and terminator – *ATPase* 3′-UTR and terminator – *RCase* coding sequence – *BSMV* 5′-UTR – *AtCAB1* promoter – *AtRbcS2B* promoter – 5′-UTR link – RNAi ds*AC1* sequence – *CaMV35S* 3′-UTR and terminator – RB

VI. Expression of the transcriptional units

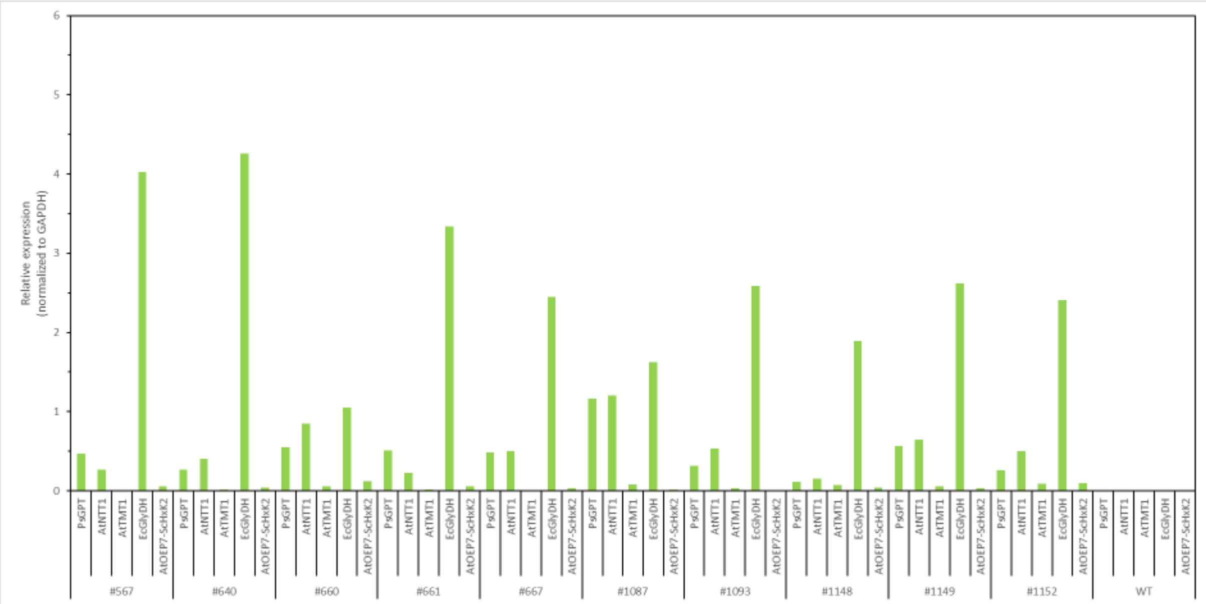

Fig. 2: Relative expression of *PsGPT*, *AtNTT1*, *AtTMT1, EcGlyDH* and *AtOEP7-ScHxK2* in leaves of the lines #567, 640, 660, 661, 667, 1087, 1093, 1148, 1149 and 1152. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

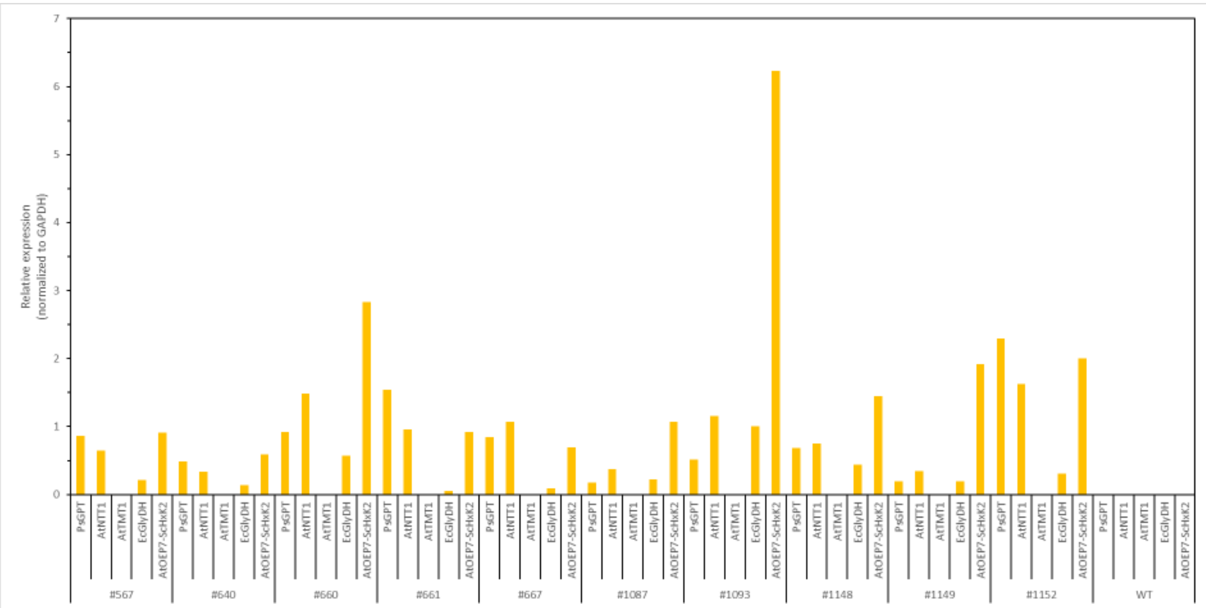

Fig. 3: Relative expression of *PsGPT*, *AtNTT1*, *AtTMT1, EcGlyDH* and *AtOEP7-ScHxK2* in roots of the lines #567, 640, 660, 661, 667, 1087, 1093, 1148, 1149 and 1152. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

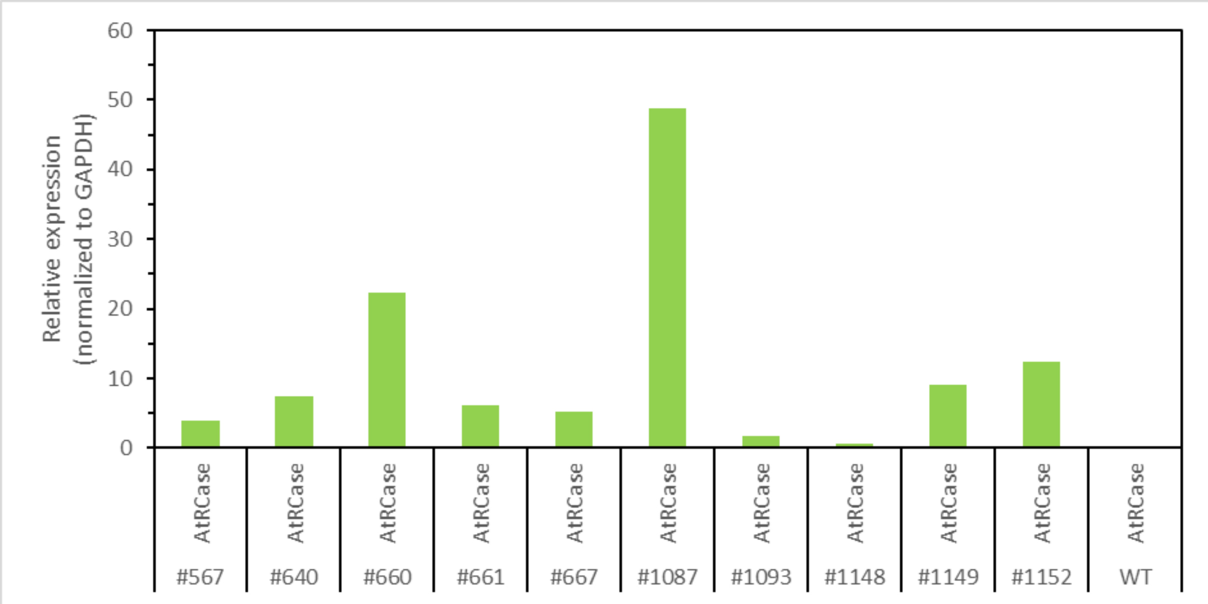

Fig. 4: Relative expression of *AtRCase* in leaves of the lines #567, 640, 660, 661, 667, 1087, 1093, 1148, 1149 and 1152. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

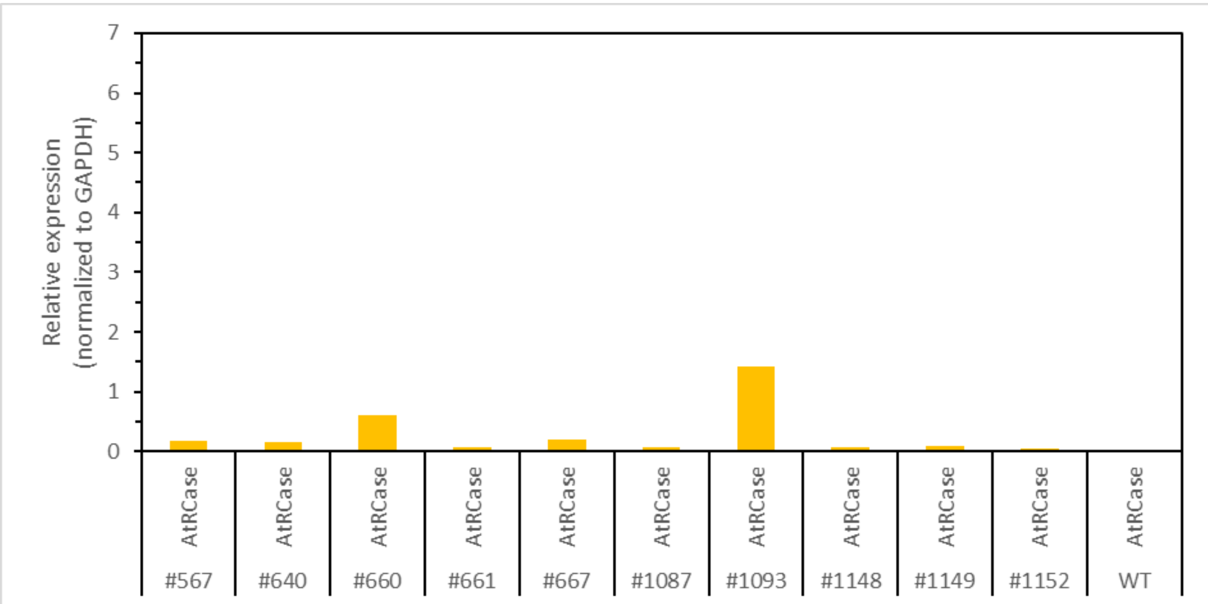

Fig. 5: Relative expression of *AtRCase* in leaves of the lines #567, 640, 660, 661, 667, 1087, 1093, 1148, 1149 and 1152. Expression values for each gene were determined with qRT-PCR, normalized to GAPDH and calculated with the delta-delta-Ct method (first descripted in Applied Biosystems User Bulletin No. 2 (P/N 4303859). No expression of transgenes was found in WT and water controls.

VII. Literature cited

Bull SE, Owiti JA, Niklaus M, Beeching JR, Gruissem W, Vanderschuren H (2009). Agrobacterium-mediated transformation of friable embryogenic calli and regeneration of transgenic cassava. *Nat Protoc* 4:1845-54.

Crafts-Brander SJ, Salvucci ME (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperatures and CO2. *Proc Natl Acad Sci U S A* 97(4):13430-435

Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu Rongda, Sederoff HW (2015). A photorespiratory bypass increases plant growth and seed yield in biofuel crop *Camelina sativa*. *Biotechnology for Biofuels* 8:175

Jonik C, Sonnewald U, Hajirezaei MR, Flügge UI, Ludewig F (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnol J* 10(9):1088-98

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge U-I (1998). Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose-6-phosphate/phosphate antiporter. *Plant Cell* 10:105-17.

Koehorst-van Putten HJJ, Wolters A-MA, Pereira-Bertram IM, van den Berg HHJ, van der Krol AR, Visser RGF (2012). Cloning and characterization of a tuberous root-specific promoter from cassava (*Manihot esculenta* Crantz). *Planta* 236:1955-65.

Kumar A, Li C, Portis AR Jr. (2009). Arabidopsis thaliana expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. *Photosynth Res.* 100(3):143-53

Nölke G, Houdelet M, Kreuzaler F, Peterhänsel C, Schillberg S (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (*Solanum tuberosum*) plastids strongly enhances photosynthesis and tuber yield. *Plant Biotechnol J*. 12(6):734-42

Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE (1998). Altered plastidic ATP/ADP-translocator activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. *Plant J* 16:531-40.

Vanderschuren H, Alder A, Zhang P, Gruissem W (2009). Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop Cassava. *Plant Mol Biol.* 70(3):265-72

Wingenter K, Schulz A, Wormit A, Wic S, Trentmann O, Hoermiller II, Heyer AG, Marten I, Hedrich R, Neuhaus HE (2010). Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in Arabidopsis. *Plant Physiol* 154:665-77.

Zhang L, Haeusler RE, Greiten C, Hajirezaei MR, Haferkamp I, Neuhaus HE, Flügge UI, Ludewig F (2008). Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. *Plant Biotechnol J* 6(5):453-64

Annex B4

Vector backbone (p134GG)

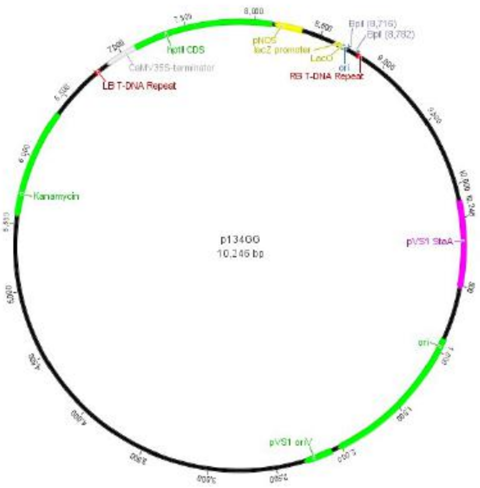

Fig.1: Vector backbone of p134GG

BpiI – recognition site of restriction enzyme type IIS BpiI, LB – left border, RB – right border, pNOS – *Nopaline Synthase* promoter, HPTII – *Hygromycin phosphotransferase* II coding sequence conferring hygromycin resistance, tCaMV35S – *Cauliflower mosaic virus* 35S 3'-UTR and terminator

Nucleotide sequence of the vector backbone p134GG:

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Legend:

RB and LB, **BpiI restriction enzyme recognition site**, Overhangs remaining after digestion with BpiI to insert functional cassettes into, NOS promoter, HPTII coding sequence, CaMV35S 3′-UTR and terminator

ANNEX C

Map of the IITA compound illustrating the habitat around the confined field.
The lake and the forest reserve are clearly well separated from the confined field.

isolation. If the breach in the reproductive isolation would be due to a cassava plant flowering inside the CFT site, the 100m isolation distance will ensure that genetic confinement is maintained.
In the unlikely event of unmanageable civil unrest or a natural disaster that affects the integrity of the CFT site, biosafety regulators will be notified and the entire experimental material will be destroyed.

7. Declaration

I hereby certify that the information in the application and all attachments is complete and accurate to the best of my knowledge and belief:

Signature of Principal Investigator for Applying Institution:

Date: 31/07/2018

Signature of Lead Scientist of Collaborating Institution:

Date: 24/07/2018

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