Agronomic and Phenotypic Characterization and Sampling of Event NUE-12 and Non-transgenic Control Rice at NaCRRI, Namulonge

2019/202 SOP for NUE-12 Regulatory Trial

1.0 Purpose and Objectives

- Establish a confined field trial(CFT) of replicated plots whose design is to characterize agronomic traits and phenotypic responses of transgenic, improved nitrogen use efficient rice (NUE)NUE-12, the relevant, non-transgenic comparator, NERICA-4, and local check variety NERICA-8 when produced in Uganda.
- During the crop field season, CFT Trial manager and technicians are to monitor, make measurements, and document plant development, pest/stress interactions (plant pests including plant disease and insect damage) and incidence of abiotic stress (drought, heat, nutrient deficiencies)within the confined field trial on a plot by plot basis.
- During the rice growing season, CFT Trial manager and technicians are to collect and procure specific rice plant samples for characterization, trait expression analysis and harvest, grain and straw samples of test (NUE-12), control (NERICA-4) and local check variety NERICA-8for subsequent analysis of composition and nutritional parameters important in rice.

2.0 Field Entries, Plant Sampling and Handling, and CFT Experimental Design

2.1 Field Entries: The established confined field trial will be strictly limited to the following three entries representing transgenic NUE-12 rice as test substance, the relevant non-transgenic rice comparator NERICA-4 as control substance, and a local rice check variety NERICA-8 as presented in Table 1. There will be five replicated blocks for a total of 15 designated plots.

Table 1. Entry Identification, Description, and Type for NUE-12 Production Plan, 2018.

Entry No.	Entry ID	Description	Type
1	NUE-12	Transgenic seed of NUE-12 in NERCIA-4 background	Test Materials
2	NERICA-4	Non-transgenic unmodified seed of parental variety NERICA-4	Control Materials
3	NERICA-8	Local check variety	Local Referenc

Regulated Seed Receipt and Inventory Storage:
A record of receipt and storage of seed to be used will be completed once seed is received. A form to facilitate process can be found in Appendix 1.

3.0 Plant Expression Samples: This production plan calls for two sampling events during the season and two sampling events at harvest (Table 2).

Table 2. Plot sampling details: time of collection, tissue types, sampling technique, and handling.

Time of Collection	Tissue Type.	Sample Definition	Plot Sampling	Handling
Early season, when thinning plots	Young Leaf Tissue	2-3 unfolded leaf stage (BBCH 13)	Five (5) leaf samples (A,B,C,D,E,F) per plot 10 grams fresh weight per plot.	Samples placed In-field coolers, conveyed to facility frozen storage prior to freeze drying& lyophilization.
Rice at 50% flowering	Whole plant	Whole aerial portion of the plant including roots.	For each plot: 3 impartially chosen plants taken from plot border rows. From each plot: sampled plants used and segmented to provide: **Roots**: 10 grams fresh weight. **Stems**:10 grams fresh weight using primary and secondary tillers without leaves. **Panicles**: 10 grams fresh weight. **Leaf samples**: from **5** impartially chosen plants[1] taken from border rows, flag leaf and leaf below flag leaf pooled together to provide 10 grams fresh wt.	Whole plants segmented to provide roots, stems (whole length without leaves) and leaf, and panicles. Samples placed In-field coolers, conveyed to facility frozen storage prior to freeze drying& lyophilization
At Harvest	Paddy rice	Mature paddy rice	For each plot: 5 impartially chosen plants taken from plot border rows. For each plot: sampled plants used to provide: Paddy Rice: 5 grams fresh weight.	Whole plants segmented to provide roots, stems (whole length without leaves) and leaf, and panicles Samples placed In-field coolers, conveyed to facility frozen storage prior to freeze drying& lyophilization
	Straw	Whole aerial portion of the plant minus the panicles	Plant Straw: 5 grams fresh weight using primary and fertile secondary tillers	

[1] With the need for 5 plants to be sampled per plot for leaf tissue, the initially chosen three plants
can be used to supply leaf samples if coupled with two additional impartially chosen plants.
Note: Prior to the off-site movement of samples, an in-field sample inventory shall be recorded in the field notebook. Prepared for use will be a material transfer form to be used to document sample identity and condition upon receipt at off-site facility. Once the material transfer form is complete please store in the field trial notebook. Appendix 2 contains an example form that facilitates process.

3.1 Rice Plant Sampling for Subsequent Compositional and Nutritional Analysis

There are two sampling events at harvest for compositional and nutritional analysis (hereafter referred to as "comp" samples). Following OECD guidelines, the two plant tissue types suggested are mature paddy rice at harvest and straw. Details can be found in Table 3 and section 2.3.1 for paddy rice, and section 2.3.2 for rice straw.

Table 3.Rice plot Comp sample details at harvest of tissue types, plot sampling, and handling.

Tissue Type.	Sample Definition	Plot Sampling	Handling
Paddy rice	Mature paddy rice	For each plot: 40 impartially chosen plants taken from plot border rows. For each plot: sampled plants used to provide: Rice panicles to be dried and threshed to render 250-300 grams of paddy rice (fresh weight at 8-10% moisture content.)	Rice panicles cut from whole plants placed in tigh mesh bags to air dry. Once dry, paddy rice thrashed free from panicles to provide paddy rice sample. Samples placed In-field containers, once air dried- packaged and conveyed to facility placed in secure, cool,dry storage prior to shipping to EPL BAS, USA.
Rice straw	Whole aerial portion of the plant minus the panicles	Rice straw: 5 grams fresh weight using primary and fertile secondary tillers	

3.1.1 Paddy Rice Comp Samples at Harvest

For each entry and on a plot by plot basis, mature panicles will be harvested from at least 40 impartially chosen representative plants from within the plot and placed into pre-labeled tight mesh sack[1]. A minimum of 250 grams of rice grain (fresh weight) is

required. Air/sun dryon-site prior to threshing mature paddy rice (target 8-10% moisture content) from the harvested panicles.
Paddy rice plot sample is to be placed into a pre-labeled[1] cloth sack which is then closed or tied securely shut to ensure containment. Transfer off-site requires double containment where plot samples are placed in a secondary container (closed lid box, or plastic tote). Once transferred to facility, plot samples need to be stored in a secure, clean (insect/vermin-free) area, under cool and dry conditions. These labelled and identity preserved samples will then be shipped to EPL Bio-Analytical Services, W. Harrison Blvd., Niantic, Ill. USA ATTN: Ms. Michelle Smith.
[1] Sample label placed inside and affixed to the outside for each sample.
Note: Prior to the off-site movement of samples, an in-field sample inventory shall be recorded in the field notebook. Prepared for use will be a material transfer form to be used to document sample identity and condition upon receipt at off-site facility. .
Once the material transfer form is complete please store in the field trial notebook. Appendix 2 contains an example form that facilitates process.

3.1.2 Rice Straw Comp Samples at Harvest.

Rice straw is defined as the above ground, aerial portions of the plant that remains after rice grain harvest. For each entry and on a plot by plot basis, the 40 plants chosen for section 2.3.1 can be used. The rice straw harvested from these 40 impartially chosen representative plants is placed into pre-labeled[1] tight mesh sacks.

Rice plants are to be cut 5-15 cm above the soil surface, any panicles removed, and the resulting straw (stem plus clasping leaves) being cut into segments small enough to fit inside redundantly pre-labelled[1] tight mesh bags which are then closed or tied to ensure sample containment. If necessary, air dryon-site to a straw moisture content of 8-10% .

A minimum of 150 grams of rice straw (dry) is required per plot. Once dried, rice strawis to be conveyed into a pre-labeled[1] cloth sack which will then be closed or tied to ensure sample containment. Once transferred to facility, plot samples need to be stored in a secure, clean (insect/vermin-free) area, under cool and dry conditions. These labelled and identity preserved samples will then be shipped to EPL Bio-Analytical Services, W. Harrison Blvd., Niantic, Ill. USA ATTN: Ms. Michelle Smith.

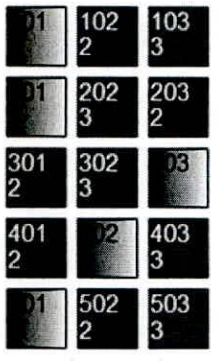
Note: Prior to the off-site movement of samples, an in-field sample inventory shall be recorded in the field notebook. Prepared for use will be a material transfer form to be used to document sample identity and condition upon receipt at off-site facility. Once the material transfer form is complete please store in the field trial notebook. Appendix 2 contains an example form that facilitates process.

3.2 Confined Field Design of NUE-12 Rice: Five blocks (replicates) of each entry (NUE-12, NERICA-4, NERICA-1) will be established in the randomized complete block design as shown in Table 4.

Table 4. NUE-12 CFT field design: plot designation by entry and within replicated blocks

Agronomic and Phenotypic Characterization of Event NUE-12 and Non-Transgenic Control Rice Grown in Africa, 2018

Trial ID:	Arcadia NUE Rice	Location:					
Protocol ID:	Arcadia NUE Rice	Investigator:					
							Replicate block 1 →
							Replicate block 2 →
Entry No.	Entry Name	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Replicate block 3 →
1	NUE-12	**101**	**201**	**303**	**402**	**501**	
2	NERICA-4	**102**	**203**	**301**	**401**	**502**	Replicate block 4 →
3	NERICA-1	**103**	**202**	**302**	**403**	**503**	
							Replicate block 5 →



[1] Sample label placed inside and affixed to the outside for each sample.

2.4.1 Plot size and plant stand establishment: Total area per each plot should be approximately ten square meters (2 meters wide, 5 meters in length) with a target stand of 250 plants per plot. Following the field design above, each entry must be hand planted in their designated plotwith ten parallel rows each five meters in length. Preferred row spacing is 20 centimeters (cm) with 20 cm between plants within each row.

For adequate plant stands and plot uniformity, rice seed for each plot will be planted in "hills"- planting spots where three seeds will be planted. After seed germination and plant emergence, hills are to be hand-thinned to a single vigorous plant per hill.

Note: It is at this time that young leaf samples are to be collected, 8 separate samples per plot (i.e. plot 101a, 101b, 101c, 101d, 101e).

After thinning, the minimum plot population is 250 plants per plot.

Disposition and devitalization of surplus thinned plants shall occur within the confines of the CFT and comply with the relevant in- country biosafety terms and conditions of authorization.

A "CFT Establishment Report" will need to be completed and submitted within 10 days of thinning operation and plot stand counts.

2.4.2 Plot identification: Each designated plot will be identified with two durable stakes (wood, plastic, or metal). Written upon the stakes will be the correct plot number (i.e. "101" and entry number (ie. "1"). The entry number shall be circled to distinguish it from the plot number. Plot stakes are to be placed at the beginning (front and center of plot) and end (back and center) of the plot.

During the entire field season, the stakes shall be maintained so that they serve their purpose in that they are present and legible. If needed, additional stakes can be made to replace broken, weathered, missing, or illegible stakes.

2.5 Plot separation: Plots within each block are to be physically separated. Unplanted alleyways should be at least 2 meters in width between designated plots. These unplanted alleyways shall remain weed-free throughout the season.

2.6 Replicated block separation: Blocks are to be separated by unplanted alleyways of at least 2 meters in width and maintained weed-free throughout the season.

2.7 Perimeter Border: A perimeter border can be planted that surrounds the regulated CFT as a physical barrier- much like a privacy fence. A perimeter border can be established with rice, cassava, maize, etc. and serves as a physical barrier as well as minimizing edge effects. The perimeter border is to be separated from the replicated blocks by unplanted alleyways of at least 1 meter in width and maintained weed-free throughout the season.

4.0 Plant Material Handling and Disposition

All plant materials established within the confined field trial shall *be handled and treated as if* regulated GM materials. In doing so, all surplus or unwanted plant materials other than the planned samples to be taken and grain saved for subsequent use as seed next season shall be disposed and devitalized on site. This includes surplus materials from the designated plots for all entries and the perimeter border. The rice, straw, and border plant materials established within the CFT are prohibited from consumption and shall not enter food/feed channels. Unauthorized, off-site movement is forbidden.

5.0 Field Data Documentation

Adequate records and timely in-season reports are critical to document progress on CFT conduct and proper management. Clear, authentic, and readily accessible records shall be maintained to document critical activities and serve to reconstruct trial conduct. Doing so serves to strengthen the linkage between on-site field observations and results, the methods used to generate reliable data, and the authenticity of plants samples subsequently used in regulatory studies that support commercial approvals required prior to farmer access.

6.0 A Field Trial Notebook (FNB) will serve as a central depository for documentation purposes and will complement this production plan.

For each site, the FNB provided includes requests for location details, site-specific conditions, training documentation of authorized personnel, specific instructions on pest/stress monitoring, data collection sheets on plant growth and development, and work instructions on sampling procedures, sample labelling, handling, and storage. As is common in laboratory notebooks, relevant observations, essential field data, and required data forms and report templates will be included.

All FNB data sheets must be filled out promptly, accurately, and legibly in indelible ink. All pages containing data must be signed (initialed/dated). If more than one individual records data on a page, it must be clear which individual recorded specific data. The original Field Data Notebook, along with all supplementary and supporting

data, is to be submitted to NBC with copies to AATF by the PI upon completion of the field report and after documentation of the date and method of crop destruction.

6.1 **Plot fertility and nutrient management:** Relevant soil analysis results for the location are needed. Nitrogen levels in the soil need to be measured to establish the base level of nitrogen already present.For the crop season, a single nutrient management regime is required where the amount of nitrogen added to the soil as a basal starter rate is to be no more than60 kilograms of total nitrogen per hectare. For example, if the base level of nitrogen already present in the soil is measured at 5 kilograms of nitrogen, then an additional 55 kilograms of nitrogen will be added and incorporated into the soil. If needed, soil amendmentsof additional macro-nutrients (e.g. phosphorus, potassium) or micro-nutrients (e.g. copper, iron) can be made based on local practices or to supply these elements based on specific rice mineral nutrition needs. All treatments should be made as uniform as possible and documented in a timely manner into the FNB. In doing so the date of application, the product used (i.e. brand name), the targeted application rate, and, if fertilizer, the concentration and form(s) of nitrogen present in the fertilizer shall be recorded.

5 Field Data on rice agronomic and plant phenotypic parameters

For each entry, field data observations and measurements will be needed onrice agronomic characteristics and plant phenotype parameters on a plot by plot basistimed specific torice growth stage (e.g. BBCH 61 onset of flowering). Table 5 lists the parameters of interest, description, and relevant growth stages appropriate for measurements or observation.

Table 5. NUE-12 Rice agronomic and phenotypic characteristics, description, and the specific rice growth stage where observations or measurements are needed.

Plant Characteristic GrowthStage[1]	Description	Rice
Seed germination	Number of viable seedlings in a randomly selected row prior to thinning unfolded[1]	11 First leaf 12 Two leaves unfolded
Plot stand 19 unfolded to unfolded	Number of plants in a randomly selected row after thinning	11- 11 First leaf 19 9th leaf
Panicle type flowering	Whole plot basis determination of panicle type (compact, intermediate, open) evaluated	BBCH 80-83 after

Panicle length Panicle width	Determine the average panicle length and width (cm) of ten randomly chosen representative plants per plot.	BBCH 85-90
Panicle number	Average panicle number per plant based on ten randomly chosen Representative plants per plot	BBCH 85-90
Flowering		
Days to first flower	Days from sowing to the date of first flower (pollen)	BBCH 61
Days to full flower	Days from sowing to >50% flowering	BBCH 61
Days to end of flowering		Days from sowing to the onset of flowering BBCH 61
Days to maturity	Number of days from sowing to physiological maturity (grain at hard dough stage)	BCCH 92
Plant height 90 DAP[1]	Distance (cm) from soil surface to tip of tallest panicle from 10 impartially chosen representative plants per plot	30, 60, and 7-10 days before harvest
Tiller number	Count the number of tillers on 10 impartially chosen representative plants per plot	30, 60, 90 DAP
Viable tillers/	Evaluated and determined on 10 impartially chosen representative plants the number of reproductively mature tillers and immature/aborted tillers.	10-14 days prior to Harvest

[1] Rice developmental stages as described in Lancashire et al. (1991) and shown in Appendix 3.
[2]A leaf is unfolded when its ligule is visible, or the tip of the next leaf is visible.

Table 5. NUE-12 Rice agronomic and phenotypic characteristics, description, and the specific rice growth stage where observations or measurements are needed (continued).

Flag leaf length/width	length and width (cm) determined from 10 impartially chosen representative plants per plot	Flag leaf 14-21 days prior to harvest
Number of spikelets	Average number of spikelets per plant based on ten impartially Chosen representative plants per plot	at or shortly after harvest
Number of filled Spikelets per plant	Average number of filled spikelets per plant based on ten randomly chosen representative plants per plot	At or shortly after harvest
Seed shattering	In plot visual estimate of the extent to of plants excessive disarticulation (loss of mature grain) through shattering.	7-10 days before harvest
Plot yield	Plot yield will be taken <u>after plot comp sampling</u> Plot yield estimates will be made by hand harvest of panicles within a 1m x 5m area being collected. Rice panicles will be threshed to procure paddy rice. For each plot, grain yield will be expressed on a normalized 14% moisture basis.	at plot harvest
Kernel weight	One thousand (1000) kernel grain weight of three (3) randomly chosen grain samples per harvested plot.	Shortly after harvest
Grain moisture	Evaluated and determined on three (3) randomly chosen samples of Grain harvested from each plot	Shortly after harvest

6 **Rice Plant Pest/Stress Monitoring, Rice Growth and Development**will include in season plant health assessments on a plot by plot basis[2]. Monitoring should begin at BBCH 11 (first leaf unfolded) and be done monthly (i.e. every 30-35 days). See Table 6 below for details.

Table 6: Rice Plant Pest/Stress, Growth and Development Parameters

Pest Observations	Scouting for damage[2] caused by **plant diseases** whole plot basis Using a 1-5 scale	Repeat every 30-25 days until harvest.
	Scouting for injury[2] caused by **insect pests** on whole plot basis Using a 1-5 scale	Repeat every 30-35 days until harvest.
Plant Stress	On a whole plot basis, scout for **stress symptoms**[2] attributed to drought, heat, and lodging using a 1-3 scale.	Repeat every 30-35 days until harvest.
Plant growth stage	pest and stress assessment, document plant growth stage using the BBCH on a plot basis.	When making Repeat every 30-35 days Until harvest.

[2]Pest and Stress Monitoring Form – with instructions for use and descriptive rating scales) shown in Appendix 4.
Form will be provided in the FNB.

Plant disease codes:	**Insect pests codes:**	**Abiotic stress codes**
RB: Rice Blast	GLH: green leafhopper	Heat: leaf curling
RsB: Rice sheath blight	BPH: brown planthopper	Drought: tiller death dry
BS: Brown spot	STB: Rice stem borer	Wind: plant lodging
Btb: Bacterial blight	ApH: plant aphids	
TnG: Tungro virus		

7.0 Field Records

7.1 Soil Characterization

Soil characterization (silty clay, loam, sandy loam, etc) should be obtained from either soil survey maps, a previous analysis, or soil samples taken for characterization with results and source documented in the FNB.

If the data is not available from a soil survey map, record how the data was obtained, and any other information that will identify how the characterization information was obtained. If a soil survey map is used, please include a high quality photocopy of the map in the FNB.

7.2 CFT Soil Fertility

An analysis of soil organic matter, nitrogen, potassium, phosphorus, calcium, magnesium, iron, copper, and manganese needs to be provided that represents CFT site soil nutrient status. Samples can be submitted for analysis with results included in the FNB.

7.3 Crop History

For the CFT site, the previous crop cultivated and harvested needs to be documented in the field notebook. If the site has no crop history, describe conditions prior to use (e.g. pasture, fallow, or ground cover weeds/grasses).

7.4 CFT Establishment Report

After planting, a CFT Establishment report needs to be provided by the Principle Investigator (or delegate CFT Trial Manager) within 12 working days to the NBC with copies to AATF. The CFT Establishment report will contain planting details and a field diagram that confirms fidelity to the CFT experimental design.

7.5 CFT Chemical Use

Treatment information for weed control (herbicides), plant disease control (fungicides) or insect control (insecticides) need to be documented in the FNB. The common name, active ingredient, formulation used, amount of formulated product used, and date of treatment needs to be included.

7.6 Sample Identification and Material Transfer Forms

Sample identification and inventory material transfer forms, once completed, are to be copied, scanned, and a photocopy or pdf scan submitted timely to NBC with copies to AATF. Completed forms need to be placed in secure storage with the FNB.

7.7 Agronomic and Phenotypic Data Recording

Field data observations and measurements on rice agronomic characteristics and plant phenotype parameters shall be recorded with the specific forms provided in the FNB. When an assessment is completed (example: seed germination), scan or otherwise copy and email pdf version to NBC with copies to AATF on a timely basis (within 7 working days of completion). Table 5 lists the parameters of interest, description, and relevant growth stages appropriate for measurements or observation.

7.8 Rice Pest, Stress, and growth/development monitoring

Plot data for each assessment of plant health/ growth and development shall be recorded with the specific forms provided in the FNB. When an assessment is completed (example: seed germination), scan or otherwise copy and email pdf version

to NBC with copies to AATF on a timely basis (within 7 working days of completion). Table 6 below provides details for each stressor and instructions regarding assessment. Forms for use can be found in the FNB.

7.9 CFT termination, crop destruction, and volunteer monitoring

Permission to terminate the CFT after harvest and destroy surplus materials need to be provided by the PI prior to CFT destruction. A termination report containing date, plot destruction details, and finality of the CFT testing phase shall be submitted by the CFT Trial Manager to the PI and NBC with copies to AATF within 7 working days upon completion. Concurrent with CFT crop destruction will be the onset of volunteer monitoring. Forms to facilitate reporting requirements will be provided in the FNB.

6.9 Seasonal and Local Weather Data

The weather data required for this plan are daily on-site rainfall, irrigation events (date, amount and source), and maximum and minimum daily air temperatures. The historical weather data required for this plan are (as a minimum) the average maximum and minimum monthly air temperatures and average monthly precipitation, obtained from the nearest national weather station.

A copy of this data, along with the field notebook, shall be submitted to NBC with copy to AATF upon completion of the field report.

Appendix 1.

Regulated Plant Seed Inventory upon Receipt Form

Sent by Name (print): **Date: / /**	**Received by Name (print):** **Signature:** **Date: / /**
Name:	Name:
Title/Organization:	Title/Organization:
Address:	Address:
Phone: Email:	Phone: Email:
CFT Study Title:	

REGULATED PLANT SEED INVENTORY UPON RECEIPT

CFT Site Code: CFT Authorization:

Entry ID	Container description (e.g. seed packets)	Weight grams	Condition upon receipt: Was seed packaging such that there was no obvious breach in containment or loss of seed? (Yes/ No[1]) Circle Response	
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /
			Yes No	Initials: Date: / /

[1] If "No": please provide details to clarify. The Comment Section form isn't required if seed packaging intact with no seed loss.

Sender Signature/ Prepared by: Date: / /

QC Performed by/QC Signature: Date: / /

Comment Section regarding Seed Receipt Inventory Information		
SENDER's Name (print): **Signature:** **Date: / /**		
Title/Organization:		
Address:		
Phone: Email:		
CFT Study Title: CFT Site Code:		
Container # or Entry ID	Container Description	Comments to clarify
		Initials: Date: / /

Appendix 2. Regulated Plant Sample Material Transfer Form

SENDER's Name (print): **Signature:** **Date: / /**	**RECEIVER's Name (print):**
Name:	Name:
Title/Organization:	Title/Organization:
Address:	Address:
Phone: Email:	Phone: Email:

REGULATED PLANT SAMPLE INVENTORY AND TRANSFER

CFT Site Code: CFT Authorization:

Entry ID	Plot #	Material Description (e.g. roots)	Weight grams	Was the plant sample, once packaged, placed in frozen storage within 5 minutes? (Yes/ No[1]) Circle Response	
NUE-12	101			Yes No	Initials: Date: / /
NUE-12	201			Yes No	Initials: Date: / /
NUE-12	303			Yes No	Initials: Date: / /
NUE-12	402			Yes No	Initials: Date: / /
NUE-12	501			Yes No	Initials: Date: / /
NERICA-4	102			Yes No	Initials: Date: / /
NERICA-4	203			Yes No	Initials: Date: / /
NERICA-4	301			Yes No	Initials: Date: / /
NERICA-4	401			Yes No	Initials: Date: / /
NERICA-4	502			Yes No	Initials: Date: / /
NERICA-1	103			Yes No	Initials: Date: / /
NERICA-1	202			Yes No	Initials: Date: / /
NERICA-1	302			Yes No	Initials: Date: / /
NERICA-1	403			Yes No	Initials: Date: / /
NERICA-1	503			Yes No	Initials: Date: / /

[1] If "No": please provide details to clarify sample handling in the Comment section provided below.
The Comment Section form isn't required if field samples are appropriately handled within the time allotted.

Prior to off-site movement of CFT samples, transport containment and label requirements must be met.

Sender Signature/ Prepared by: Date: / /

QC Performed by/QC Signature: Date: / /

Comment Section regarding Sample Inventory and Transfer Information			
SENDER's Name (print): **Signature:**		**Date: / /**	
Title/Organization:			
Address:			
Phone:		Email:	
CFT Study Title: CFT Site Code:			
Entry ID	Plot #	Description (e.g. roots)	Comments to clarify
			Initials: Date: / /
			Initials: Date: / /
			Initials: Date: / /
Prepared by (print) :			Signature: /
Date: / /			

Appendix 3. Phenological plant growth stages with BBCH identification keys for rice (*Oryza sativa* L.)[1]

Principal Growth Stage	Code and Description
0 Germination	00 Dry seed (caryopsis) 01 Beginning of seed imbibition 03 Seed imbibition complete (pigeon breast) 05 Radicle emerged from caryopsis 06 Radicle elongated, root hairs and/or side roots visible 07 Coleoptile emerged from caryopsis (in water-rice this stage occurs before stage 05) 09 Imperfect leaf emerges (still rolled) at the tip of the coleoptile
1. Leaf development	10 Imperfect leaf unrolled, tip of first true leaf visible 11 First leaf unfolded 12 Two leaves unfolded 13 Three leaves unfolded 14-18Number of leaves unfolded until. 19 Nine or more leaves unfolded
2. Tillering[b]	21 Beginning of tillering: first tiller detectable 22 2 tillers detectable 23 3 tillers detectable 2 . Stages continuous till ... 29 Maximum number of tillers detectable
3. Stem elongation[c]	30 Panicle initiation or green ring stage: chlorophyll accumulates in the stem tissue, forming a green ring 32 Panicle formation: panicle 1–2 mm in length 34 Internode elongation or jointing stage: internodes begin to elongate, panicle more than 2 mm long (variety-dependent) 37 Flag leaf just visible, still rolled, panicle moving upwards 39 Flag leaf stage: flag leaf unfolded, collar regions (auricle and ligule) of flag leaf and penultimate leaf aligned (pre-boot stage)
4. Booting	41 Early boot stage: upper part of stem slightly thickened, sheath of flag leaf about 5 cm out of penultimate leaf sheath 43 Mid boot stage: sheath of flag leaf 5–10 cm out of the penultimate leaf sheath 45 Late boot stage: flag leaf sheath swollen, sheath of flag leaf more than 10 cm out of penultimate leaf sheath 47 Flag leaf sheath opening 49 Flag leaf sheaths open
5. Inflorescence emergence, heading	51 Beginning of panicle emergence: tip of inflorescence emerged from sheath 52 20% of panicle emerged 53 30% of panicle emerged 54 40% of panicle emerged 55 Middle of panicle emergence: neck node still in sheath 56 60% of panicle emerged 57 70% of panicle emerged

	58 80% of panicle emerged 59 End of panicle emergence: neck node level with the flag leaf auricle, anthers not yet visible
6. Flowering, anthesis	61 Beginning of flowering: anthers visible at top of panicle 65 Full flowering: anthers visible on most spikelets 69 End of flowering: all spikelets have completed flowering but some dehydrated anthers may remain
7. Development of fruit	71 Watery ripe: first grains have reached half their final size 73 Early milk 75 Medium milk: grain content milky 77 Late milk
8. Ripening	83 Early dough 85 Soft dough: grain content soft but dry, fingernail impression not held, grains and glumes still green 87 Hard dough: grain content solid, fingernail impression held 89 Fully ripe: grain hard, difficult to divide with thumbnail
9. Senescence	92 Over-ripe: grain very hard, cannot be dented by thumbnail 97 Plant dead and collapsing 99 Harvested product

From BBCH monograph based on Lancashire et al., 1991.

[a]A leaf is unfolded when its ligule is visible or the tip of the next leaf is visible.

[b] Tillering or stem elongation may occur earlier than stage 13; in this case continue with stages 21 or 30

[c] If stem elongation begins before the end of tillering continue with stage 30.

Appendix 4.

Rice Pest and Stress Monitoring Form

Instructions for use: Use this field monitoring form throughout each season. Print or photocopy as many as necessary prior to use. Please enter the requested information directly and promptly. Entries need to be clear and legible.

Consider what follows as guidelines while making field observations.

Starting 14-21 days after planting and repeated every 30-35 days thereafter thru to harvest, each plot will be examined for rice plant pests (disease damage and insect injury) and abiotic stress using the Rice Pest and Stress Monitoring Form.

For each plot gauge overall plant health and rice plant growth and development. Examine plants displaying injury (disease/insect damage), whole plant stress or altered, abnormal

growth. Document any discernable injury or damage caused by disease or insects. Likewise, on altered and abnormal growth.

Adverse Environmental Effects: If severe deleterious or negative effects are suspected within the CFT plants, the surrounding plants, or on non-targets species, or the environment enter your observations into the CFT Log section and notify the Principal Investigator (PI) at once. You may be asked to make additional observations and/or provide pictures as directed by the PI.

This is especially important when transgenic plants consistently appear to be markedly different from their non-transgenic controls with regards to stress/ pest interactions or adverse effects to the environment. Document to the extent necessary and note the plant phenotypes observed.

Plant disease codes:	**Insect pests codes:**	**Abiotic stress codes**
RB: Rice Blast	STB: Rice stem borer	Heat: leaf curling
RsB: Rice sheath blight	ApH: plant aphids	Drought: tiller death dry
BS: Brown spot		Wind: plant lodging
Btb: Bacterial blight		
RYMVTungro virus		

		Rice plant diseases (1-5)					Rice insect pest (1-5)		Abiotic stress (1-3)		
Entry	Plot	RB	RSB	BS	Btb	Tng	StB	AphH	drought	wind	Rice
ID	#										GS[1]
NUE-12	101										
NERICA-4	102										
NERICA-8	103										
NUE-12	201										
NERICA-1	202										
NERICA-4	203										
NERICA-4	301										
NERICA-1	302										
NUE-12	303										
NERICA-4	401										
NUE-12	402										
NERICA-1	403										
NUE-12	501										
NERICA-4	502										
NERICA-1	503										

Answer the following questions by circling either "yes" or "no":

Any evidence of harmful effects with the transgenic plants?

Yes[1]/No (circle one).

Has crop development, especially with regards to the transgenic plants, occurred as expected and is acceptable?

Yes[1]/No (circle one)

[1]**If you responded yes to either question- please explain and describe in the CFT Log and notify PI.**

Plot plant disease ratings

Field reaction of test entries to plant diseases will be recorded on a 1-5 scale described as follows:

Score	Plant disease Incidence/Severity
1	Clean, no infection, no observed symptoms
2	Few (< 2%) and scattered affected plants with mild symptoms.
3	Obvious (>5-20% disease) with moderate symptoms.
4	Obvious (>21-50%) but with severe symptoms.
5	Pronounced (>50%) with severe symptoms, tiller abortion, premature plant.

Plot insect pest evaluations

Field reaction of test entries to insect pest damage will be recorded using the following 1-5 scale:

Index	Insect Damage Assessment Description
1	No signs of infestation or damage.
2	Scattered few plants with slight yellowing due to feeding. Insect pressure low.
3	Obvious leaves partially yellow. Insect pressure moderate.
4	Leaves with pronounced yellowing and some stunting or wilting. Insect pressure high with action threshold (i.e. insecticide) exceeded.
5	More than half the plants wilting and/or remaining plants severely stunted. Insect pressure high.

Plot Abiotic Stress assessments

Field reaction of test entries to common stress factors will be recorded using a 1-3 evaluation scale.

Index	Insect Damage Assessment Description
1	No signs of stressor.
2	Moderate symptoms of stress with minor impact on plant growth and development.
3	Severe symptoms of excessive stress reducing plant growth and development affecting overall yield.

Rice Plant Disease Symptomology

Rice Sheath Blight: Grayish green lesions that enlarge and coalesce to form blotches mostly on the sheath.

Rice blast: Leaf lesions are spindle-shaped with brown borders and grayish centers. Evaluate plants at 27-53 days after transplanting (DAT) or at growth stages 3-5 on the first to fourth leaves from base of the plant.

Brown spot: Lesions on leaves are brown at center, oval, relatively uniform, and eventually distributed over the leaf surface. Fully developed spots on glumes have brown or whitish centers. Evaluation is similar to that of narrow brown leaf spot.

Bacterial blight : Undulated lesions near the leaf tips or margins extending downward. Young lesions are light green to grayish green, turning yellow to gray with time.

RYMV: Mottled and yellow to yellow-orange leaves, stunted growth and slightly reduced tillering.

Rice Insect Pests

Rice Stem borer (STB): The larva of rice stem borer tunnel into rice stem and hollow out stem completely, creating dead heart symptoms during vegetative stage of rice and whitehead symptoms during reproductive growth stage of rice.