

Field trial of *Xanthomonas* wilt disease-resistant bananas in East Africa

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Supplementary Methods

Plant preparation for field trial

Transgenic banana lines expressing sweet pepper *Hrap* or *Pflp* gene were generated and analyzed as described by Tripathi *et al.*¹ and Namukwaya *et al.*² respectively. Sixty five transgenic lines (40 lines expressing *Hrap* gene and 25 lines with *Pflp* gene) which have shown 100% resistance against *Xanthomonas campestris* pv. *musacearum* (*Xcm*) in glasshouse experiments using potted plants, the presence of low copies of the transgene and detectable expression of expected RNA signals, were selected for field evaluation. Transgenic and nontransgenic control plantlets were micropropagated on proliferation medium [MS salts and vitamins³, 10 mg/l ascorbic acid, 100 mg/l myo-inositol, 5 mg/l BAP, 30 g/l sucrose, 3 g/l gelrite, pH 5.8]. The individual shoots were transferred to rooting medium (MS salts and vitamins, 10 mg/l ascorbic acid, 100 mg/l myo-inositol, 1 mg/l IBA, 30 g/l sucrose, 3 g/l gelrite, pH 5.8). The well-rooted transgenic and nontransgenic plants were weaned in small disposable plastic cups (10 cm) containing sterile soil and placed under transparent polythene chamber with diffused light, high humidity and 26-28°C in a contained (Biosafety level II) glasshouse. Plants were acclimatized for 4 weeks under polythene chamber where humidity was reduced over time by gradual opening the side of the chamber in the third week. Subsequently, after 4 weeks plants were removed from the polythene chamber and transferred to bigger pots (30 cm) in the glasshouse. Plants were irrigated manually on alternate days. Three months old plants were used for planting in confined field.

Field planting, plot design, maintenance and harvesting

A replicated trial of 65 transgenic lines of banana cv. 'Sukali ndiizi' (apple banana; AAB group) and 'Nakinyika' (East Africa Highland banana; AAA group), along with control nontransgenic plants of both cultivars were planted in October 2010 using randomized block design in a confined field at the National Agriculture Research Laboratories, Kawanda, Uganda. The site had been previously approved by the Ugandan National Biosafety Committee, authorization code: 2/2010. The field was prepared by ploughing twice with tractor. Three months old plants were removed from their pots and planted into 30 cm wide, 30 cm deep holes and backfilled with top soil with plant spacing of 3 m X 3 m. A randomized complete block experimental design was followed with three replications. One replicate of each transgenic line was planted in each block and there were three blocks. Five replicates of nontransgenic controls

were planted in each block. The reference wild type, uncultivated, not edible variety *Musa balbisiana* (BB group) previously reported to be resistant to BXW^{4,5,6,7}, was also included in the trial. Tissue culture plants of same size were planted to form a border around and between each experimental plot. Plants were watered daily for 1 month after planting and three times per week thereafter until plants established. After that plants were irrigated naturally through rain.

The trial was managed using recommended farming practices (weeding, de-suckering, mulching, water holding trenches etc.). Hand weeding and de-suckering was done on monthly basis. No fungicide or nematicide was applied. Following formation of the bunch, the male flowers were removed. The emerging fruit bunches along with the flowers were bagged after appearance of flowers until male flowers were removed. The phenotype was observed by visual inspection for structural abnormalities and data were collected on agronomic performance. The plants were evaluated for two successive crop cycles (mother and ratoon). The trial was terminated in December 2012.

BXW disease assessment

The mother and first ratoon plants of transgenic lines and nontransgenic lines were evaluated for BXW resistance by artificial inoculation as described by Tripathi *et al.*¹. For BXW resistance screening, pure cultures of *Xcm* isolated from the infected plants, for which Koch's postulate had been proved, were maintained on Y TSA medium (1% yeast extract, 1% tryptone, 1% sucrose and 1.5% agar) at 4 °C. A single bacterial colony was inoculated into 25 ml of YTS medium (1% yeast extract, 1% tryptone, and 1% sucrose) and cultured at 28 °C with shaking at 150 rpm for 48 h. The bacterial culture was centrifuged at 5000 rpm for 5 min and pellet was re-suspended in sterile double distilled water. The optical density (OD 600 nm) of the bacterial suspension was checked and bacterial concentration was adjusted to 10⁸ cfu/ml with sterile water. Fresh inoculum was used for all the experiments in order to have high virulent potential of the pathogen. The bacterial suspension (1 ml) was injected into the midrib of first fully open leaf of mature, preflowering-stage plants of mother and ratoon crops using a syringe (5 ml) fitted with a 24 gauge needle. Three plants of each transgenic plant, nine plants (3 in each block) of control nontransgenic plants and three plants of reference variety '*Musa balbisiana*' were inoculated. Six plants of control nontransgenic plants were left un-inoculated. The inoculated plants were assessed every day for disease symptoms with preliminary symptoms as chlorosis or necrosis of leaves and finally as complete wilting of plants, until harvest time. At harvesting, the number of leaves wilted were counted and also internal symptoms in pseudostem, rachis and fruits were observed and disease severity was scored on the scale of 0-5 [0- no symptoms, 1- only one leaf wilted, 2- 2 to 3 leaves wilted, 3- 4 to 5 leaves wilted, 4- all the leaves wilted but plant still alive, 5- whole plant died].

The relative resistance of transgenic plants to BXW was evaluated at harvest time based on reduction in wilting in comparison to control nontransgenic plants.

Resistance (%) = (Reduction in wilting of transgenic plant / Mean wilting in control nontransgenic plants) X 100;

Reduction in wilting= (Mean proportion of leaves wilted in control nontransgenic plants) – (proportion of leaves wilted in transgenic plant).

The pathogenic bacteria were re-isolated from the pseudostem of all the inoculated plants as described by Tripathi *et al.*⁸ for identification as *Xcm* on the basis of their characteristic morphology as yellowish, mucoid and circular colonies and by PCR analysis using *Xcm* specific primers⁹. The non-symptomatic plants were also tested with PCR using plant tissue for confirmation.

All the lines have unique codes and artificial inoculations and scoring were both carried out blindly by one person, who was not involved in the development, molecular characterization and glasshouse screening of transgenic lines.

Data collection for agronomic and yield performance

Data on plant height, girth and number of functional leaves were collected at flowering and harvest. Time for flowering and harvesting of fruits was also recorded. At harvest, data on bunch weight, number of hands, number of fingers and weight of individual fruit were collected. Data collected for transgenic plants were compared with un-inoculated nontransgenic plants of same varieties.

PCR analysis

PCR analysis was performed with all the plants grown in field to check for the stability of gene. Genomic DNA was isolated from the field grown transgenic mother plants and ratoon plants using DNeasy kit (Qiagen, GmbH, Hilden, Germany). PCR analysis was performed using gene specific primers to confirm presence of transgenes into the plant genome. The primer sequences were *Pflp* gene specific primers: Forward 5'CAAGAAAACCAGCTGTGACAAGCCTTAAAC3' and Reverse 5'CGAGTTCTGCCTCTTTGTGAGTCTCAATAG3' and *Hrap* specific primers: Forward 5'GAGCTCACAGCATTTTGGCCATCCC3' and Reverse 5'TGGAGTTGGAGGACGAGGAAC3'. PCR was performed in 25 µl reaction mixture containing 1.5 mM MgCl₂, 1 X reaction buffer, 0.2 mM nucleotide mix, 1 µM primers, 1 Unit of Taq DNA Polymerase and 1 µg of template DNA. PCR products were resolved by electrophoresis on 0.8% agarose gel and visualized under UV radiation.

RNA extraction, RT-PCR and qRT-PCR analysis

RNA was extracted from leaf tissue of the entire field grown transgenic lines and control nontransgenic plant as described by Yang *et al.*¹⁰. The RNA samples were treated with RNase-free DNase (Fermentas, Hanover, MD, USA). Following standard phenol/chloroform extraction, RNA was precipitated by adding 0.1 volume of sodium acetate (3 M) and 2 volumes of ethanol 100% and incubating at -20°C for 2 hrs. The RNA was recovered by centrifugation (13,000 rpm for 15 min at 4°C), washed with 70% ethanol, air dried and then dissolved in 40 µl of RNase-free

water. The quantity and quality (A260/230 and A260/280) of total RNA were determined using the Nanodrop. RNA was checked with PCR for absence of genomic DNA. First-strand cDNA was synthesized using 2 µg DNase-treated RNA, 1 µM OligodT (18 mer), 1× RT buffer, 10 µM dNTP mix (Fermentas) and 200 U Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT; Fermentas). RT-PCR of all the *Pflp* lines was performed using *Pflp* gene specific primers (Forward 5'GAGCTCCCAAACGTTGGGGAAGC3' and Reverse 5'ACGAGTTCTGCCTCTCTTTGTAGT3') and all *Hrap*-lines was performed with *Hrap* specific primers (Forward 5'GAGCTCACAGCATTTTGGCCATCCC3' and Reverse 5'TGGAGTTGGAGGACGAGGAAC3'). Amplification of the *Musa 25S* ribosomal gene, used as an internal control to determine the quality of RNA, was performed using the forward primer (forward primer: 5' ACATTGTCAGGTGGGGAGTT 3'; reverse primer: 5' CCTTTTGTTCACACGAGATT 3').

Fourteen transgenic lines (7 *Hrap* lines showing 100% resistance, 2 *Hrap* lines showing partial resistance, 3 *Pflp* lines showing 100% resistance and 2 *Pflp* lines showing partial resistance) were further analyzed for gene expression using qRT-PCR. The qRT-PCR was carried on 7900 Real Time PCR System (Applied Biosystems, USA) using Maxima SYBR green/ROX PCR kit (Thermo Scientific, USA) according to the manufacturer's instructions. qRT-PCR was performed with 1 µl of each cDNA synthesized at 1:10 dilution, using gene specific primers (*Hrap* forward primer 5'TCTGATGCAATTGGAGGAGGAA3'; *Hrap* reverse primer 5'ACGGTTGTAAGTCTGTGAGT3'; *Pflp* forward primer 5'TCCTTCATGCCAAGAAAACC3' and *Pflp* reverse primer 5'TGGTCCGTCAGGTGTGATAA3'). Two independent biologically replicated experiments were set up with three technical replicates in each experiment. No-template controls and nontransgenic control were included in each experiment. Relative expression data were normalized using the *Musa 25S* ribosomal gene. Nontransgenic control plant acts as calibrator to calculate relative expression level of *Hrap* or *Pflp* in transgenic plants. The relative levels of *Hrap* or *Pflp* gene were analyzed using the $2^{-\Delta\Delta C_t}$ method¹¹.

ΔC_t (*Hrap* or *Pflp*) = C_t (*Hrap* or *Pflp*) – C_t (internal control 25S ribosomal gene)

$\Delta\Delta C_t$ = ΔC_t (target sample or transgenic line) – ΔC_t (calibrator or nontransgenic plant)

Statistical analysis

All the data collected were analyzed using mixed model procedure of SAS¹², with treatment combinations being fixed and replicates as random effects. The cluster analysis was performed on the disease data using the CLUSTER procedure of SAS¹². The standardized variables (mean=0; variance=1) were subjected to Ward's minimum variance method of the cluster procedure^{13,14}. The output was printed to display the cluster membership as well as dendrogram of the transgenic lines. Also analysis of variance (ANOVA) was performed on the agronomic variables measured (number of functional leaves, plant height at flowering, number of days for flowering; girth of pseudostem; bunch weight; number of hands per bunch; number of fruits per bunch and average weight of each fruit) to compare the means of transgenic lines with means of control nontransgenic plants. Furthermore, correlation analysis was performed to investigate the linear association or relationship between the disease variables and the agronomic traits.

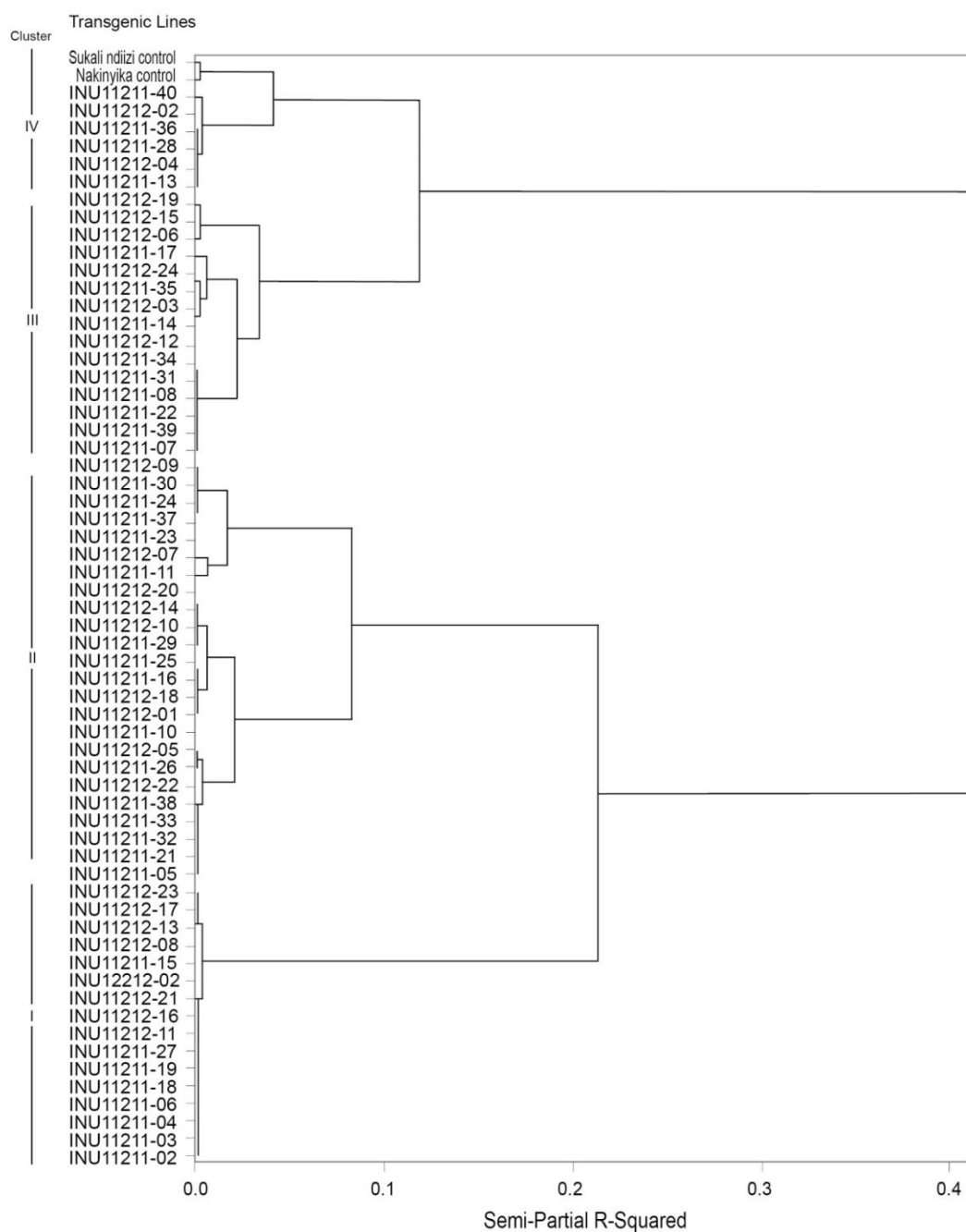
References

1. Tripathi, L., Mwaka, H., Tripathi, J.N. & Tushemereirwe, W.K. *Mol. Plant Pathol.* **11**, 721-731 (2010).
2. Namukwaya, B., *et al.* *Transgenic Res.* **21**, 855-865 (2012).
3. Murashige, T. & Skoog, F. *Physiol. Plant.* **15**, 473-497 (1962).
4. Kumakech, A., Kiggundu, A. & Okori, P. *Afr. Crop Sci. J.* **21**, 337 – 346 (2013).
5. Ssekiwoko, F., Tushemereirwe, W.K., Batte, M., Ragama, P.E. & Kumakech, A. *Afr. Crop Sci. J.* **14**, 151-155 (2006).
6. Tripathi L. & Tripathi J.N. *Afr. J. Biotechnol.* **8**, 5343-5350 (2009).
7. Tripathi, L. *et al.* *Plant Dis.* **93**, 440–451 (2009).
8. Tripathi, L., Tripathi, J.N., Tushemereirwe, W.K. & Bandyopadhyay, R. *Eur. J. Plant Pathol.* **117**, 177–186 (2007).
9. Adikini, S., *et al.* *Plant Pathol.* **60**, 443-452 (2011).
10. Yang, G., Zhou, R., Tang, T. & Shi, S. *Prep. Biochem. Biotechnol.* **38**, 257-264 (2008).
11. Livak, K.J. & Schmittgen, T.D. *Methods* **25**, 402–408 (2001).
12. SAS/STAT 9.3 User's Guide, SAS Institute, Cary, NC (2012).
13. Anderberg, M. R. *Cluster Analysis for Applications*, New York: Academic Press (1973).
14. Ward, J.H. JR., *J. Am. Stat. Assoc.* **58**, 236-244 (1963).

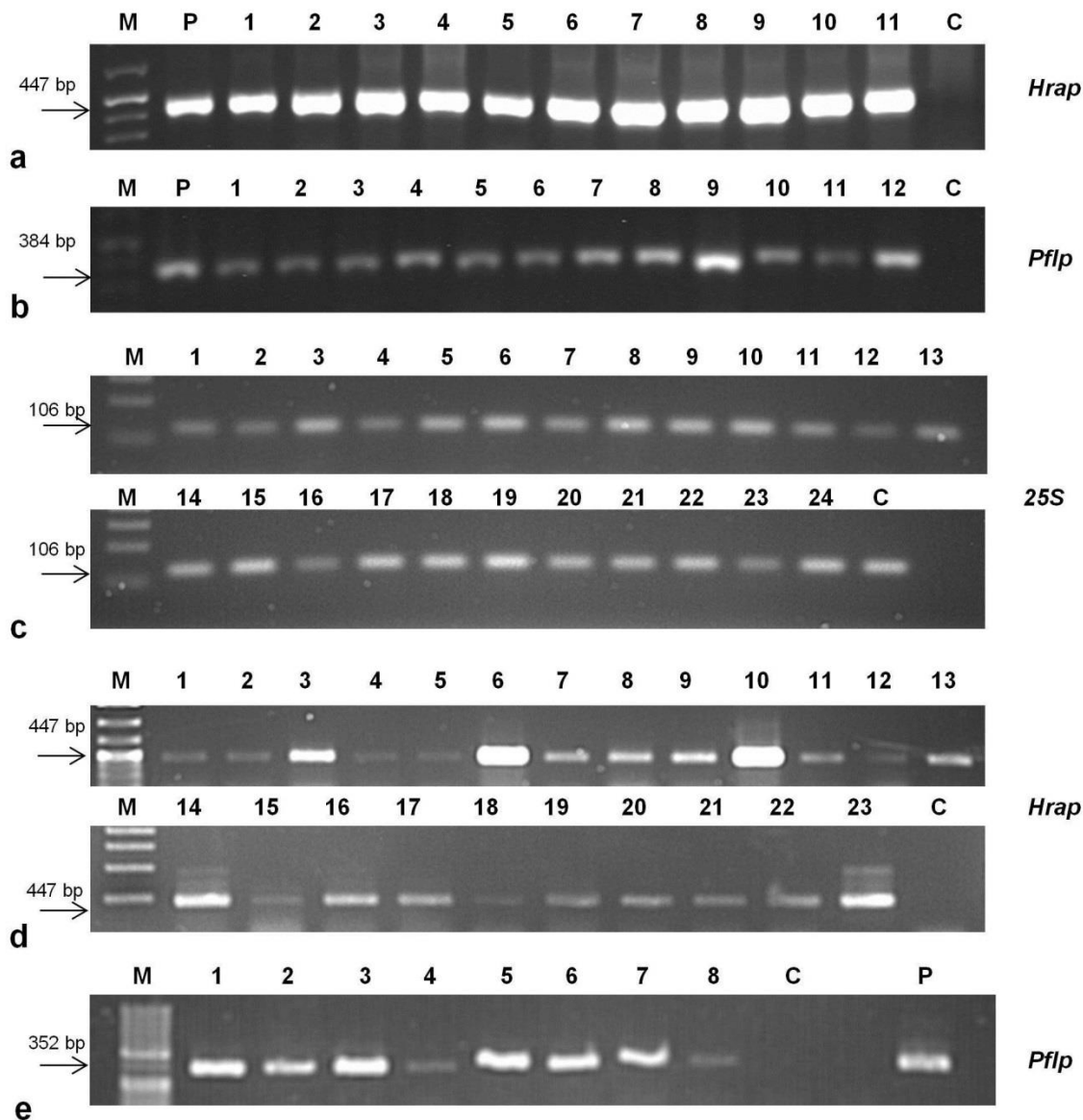
Supplementary figures



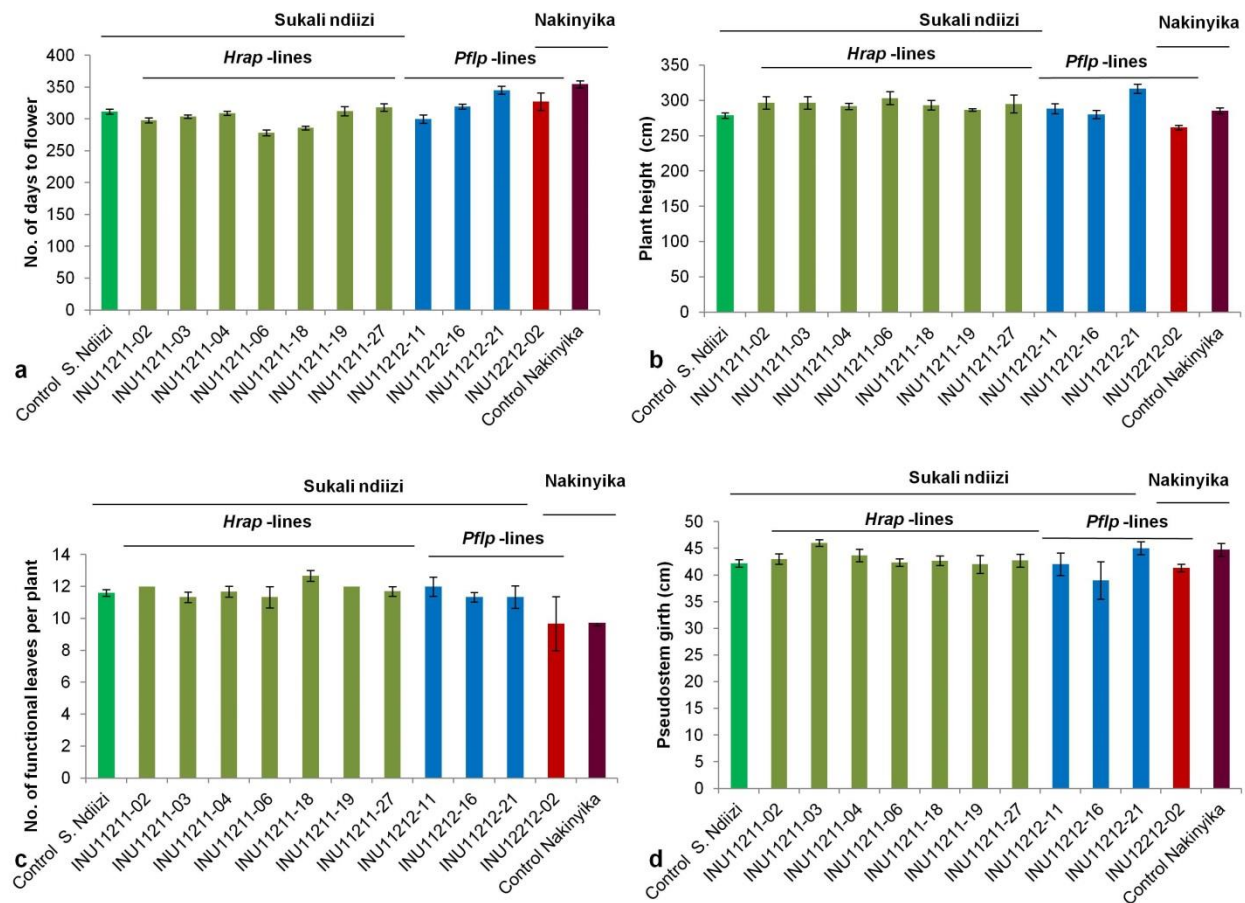
Supplementary Figure 1 Transgenic plants showing normal morphology in the confined field trial.



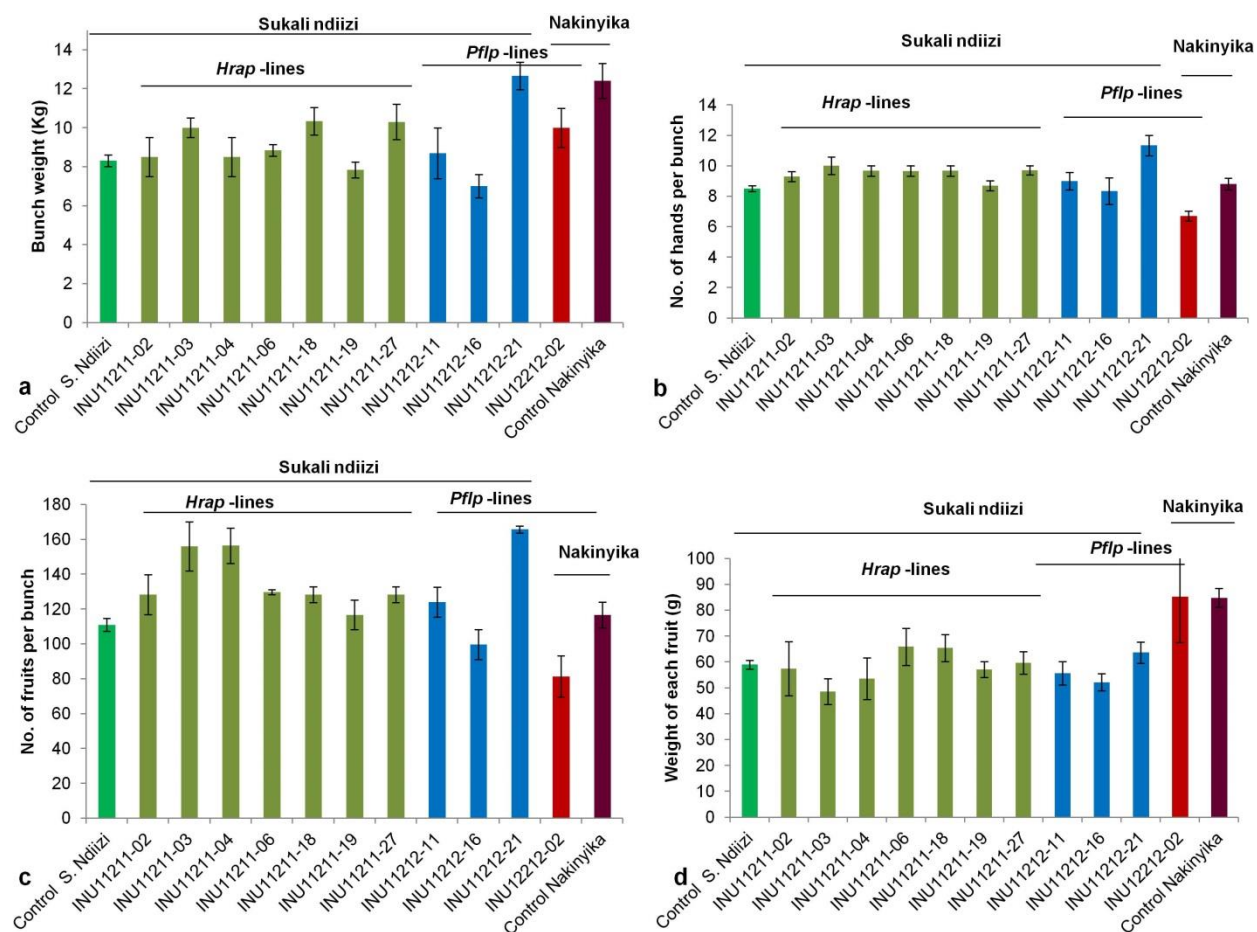
Supplementary Figure 2 Dendrogram produced from Ward's minimum variance cluster analysis showing disease resistance of transgenic lines in comparison to nontransgenic control plants evaluated under field conditions.



Supplementary Figure 3 Representative picture of PCR analysis of genomic DNA from different transgenic lines and nontransgenic plant using gene specific primers. **(a)** *Hrap* specific primers; **(b)** *Pflp* specific primers; M- molecular weight marker; P- plasmid vector DNA; 1-12- transgenic lines; C- control nontransgenic plant. Representative picture of RT-PCR analysis of RNA isolated from leaf tissue using gene specific primers. **(c)** *Musa 25S* ribosomal gene specific primers as internal control; **(d)** *Hrap* specific primers; **(e)** *Pflp* specific primers. P- plasmid construct DNA; 1-25- transgenic plants; C- nontransgenic plants.



Supplementary Figure 4 Growth performance of the best eleven BXW resistant transgenic lines in comparison to nontransgenic plants. **(a)** Number of days to flower; **(b)** Plant height at maturity; **(c)** Number of functional leaves per plant at flowering; **(d)** Pseudostem girth of mature plant. Results are presented as score mean $\pm$ S.E. based on three replicates.



Supplementary Figure 5 Yield performance of the best eleven BXW resistant transgenic lines in comparison to nontransgenic plants. **(a)** Bunch weight of each plant; **(b)** Number of hands per bunch; **(c)** Number of fruits per bunch; **(d)** Weight of each fruit. Results are presented as score mean $\pm$ S.E. based on three replicates.

Supplementary Table 1: BXW disease assessment of transgenic lines in comparison to control nontransgenic lines.

Line Code	Cultivar	Transgene	Disease severity (0-5)		Resistance (%)		Cluster
			Mean*± S.E. Mother	Ratoon	Mean*± S.E. Mother	Ratoon	
INU11211-02	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-03	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-04	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-06	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-15	Sukali ndiizi	<i>Hrap</i>	0.3 ± 0.3	0.0 ± 0.0	88.9 ± 11.1	100.0 ± 0.0	1
INU11211-18	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-19	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-27	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11212-08	Sukali ndiizi	<i>Pflp</i>	0.3 ± 0.3	0.0 ± 0.0	91.7 ± 8.3	100.0 ± 0.0	1
INU11212-11	Sukali ndiizi	<i>Pflp</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11212-13	Sukali ndiizi	<i>Pflp</i>	0.3 ± 0.3	0.0 ± 0.0	93.3 ± 6.7	100.0 ± 0.0	1
INU11212-16	Sukali ndiizi	<i>Pflp</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11212-17	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.7	0.0 ± 0.0	91.7 ± 8.3	100.0 ± 0.0	1
INU11212-21	Sukali ndiizi	<i>Pflp</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11212-23	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.7	0.0 ± 0.0	85.2 ± 14.8	100.0 ± 0.0	1
INU12212-02	Nakinyika	<i>Pflp</i>	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	1
INU11211-05	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11211-10	Sukali ndiizi	<i>Hrap</i>	0.7 ± 0.7	1.7 ± 1.7	83.3 ± 16.7	66.7 ± 33.3	2
INU11211-11	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	5.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	2
INU11211-16	Sukali ndiizi	<i>Hrap</i>	1.0 ± 0.0	1.7 ± 1.7	88.0 ± 0.5	66.7 ± 33.3	2
INU11211-21	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11211-23	Sukali ndiizi	<i>Hrap</i>	0.3 ± 0.3	3.3 ± 1.7	95.2 ± 4.8	33.3 ± 33.3	2
INU11211-24	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	3.3 ± 1.7	100.0 ± 0.0	33.3 ± 33.3	2
INU11211-25	Sukali ndiizi	<i>Hrap</i>	0.7 ± 0.7	1.7 ± 1.7	87.5 ± 12.5	66.7 ± 33.3	2
INU11211-26	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	2.3 ± 1.4	100.0 ± 0.0	50.0 ± 28.9	2
INU11211-29	Sukali ndiizi	<i>Hrap</i>	1.7 ± 1.7	1.7 ± 1.7	55.6 ± 29.4	66.7 ± 33.3	2
INU11211-30	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	3.3 ± 1.7	100.0 ± 0.0	33.3 ± 33.3	2
INU11211-32	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11211-33	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11211-37	Sukali ndiizi	<i>Hrap</i>	0.3 ± 0.3	3.3 ± 1.7	93.3 ± 6.7	33.3 ± 33.3	2
INU11211-38	Sukali ndiizi	<i>Hrap</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11212-01	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.3	1.7 ± 1.7	79.4 ± 10.4	66.7 ± 33.3	2
INU11212-05	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.7	2.0 ± 1.5	85.7 ± 14.3	50.0 ± 28.9	2
INU11212-07	Sukali ndiizi	<i>Pflp</i>	1.7 ± 1.7	5.0 ± 0.0	66.7 ± 33.3	0.0 ± 0.0	2
INU11212-09	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.7	3.3 ± 1.7	85.2 ± 14.8	33.3 ± 33.3	2
INU11212-10	Sukali ndiizi	<i>Pflp</i>	1.7 ± 1.7	1.7 ± 1.7	66.7 ± 33.3	66.7 ± 33.3	2
INU11212-14	Sukali ndiizi	<i>Pflp</i>	1.7 ± 1.7	1.7 ± 1.7	66.7 ± 33.3	66.7 ± 33.3	2

INU11212-18	Sukali ndiizi	<i>Pflp</i>	0.7 ± 0.7	1.7 ± 1.7	73.3 ± 26.7	66.7 ± 33.3	2
INU11212-20	Sukali ndiizi	<i>Pflp</i>	1.3 ± 0.7	1.7 ± 1.7	71.4 ± 16.5	66.7 ± 33.3	2
INU11212-22	Sukali ndiizi	<i>Pflp</i>	0.0 ± 0.0	1.7 ± 1.7	100.0 ± 0.0	66.7 ± 33.3	2
INU11211-07	Sukali ndiizi	<i>Hrap</i>	2.3 ± 1.4	1.7 ± 1.7	52.4 ± 29.0	66.7 ± 33.3	3
INU11211-08	Sukali ndiizi	<i>Hrap</i>	2.7 ± 1.2	1.7 ± 1.7	47.7 ± 25.6	66.7 ± 33.3	3
INU11211-14	Sukali ndiizi	<i>Hrap</i>	4.0 ± 1.0	1.7 ± 1.7	25.0 ± 25.0	66.7 ± 33.3	3
INU11211-17	Sukali ndiizi	<i>Hrap</i>	5.0 ± 0.0	1.7 ± 1.7	0.0 ± 0.0	66.7 ± 33.3	3
INU11211-22	Sukali ndiizi	<i>Hrap</i>	2.3 ± 1.3	1.7 ± 1.7	47.2 ± 23.7	66.7 ± 33.3	3
INU11211-31	Sukali ndiizi	<i>Hrap</i>	2.7 ± 1.4	1.7 ± 1.7	48.1 ± 28.9	66.7 ± 33.3	3
INU11211-34	Sukali ndiizi	<i>Hrap</i>	2.7 ± 1.4	1.7 ± 1.7	45.8 ± 29.2	66.7 ± 33.3	3
INU11211-35	Sukali ndiizi	<i>Hrap</i>	3.3 ± 1.7	1.7 ± 1.7	33.3 ± 33.3	66.7 ± 33.3	3
INU11211-39	Sukali ndiizi	<i>Hrap</i>	2.3 ± 1.4	1.7 ± 1.7	55.6 ± 29.4	66.7 ± 33.3	3
INU11212-03	Sukali ndiizi	<i>Pflp</i>	4.0 ± 1.0	1.7 ± 1.7	20.8 ± 20.8	66.7 ± 33.3	3
INU11212-06	Sukali ndiizi	<i>Pflp</i>	1.7 ± 1.7	0.0 ± 0.0	66.7 ± 33.3	100.0 ± 0.0	3
INU11212-12	Sukali ndiizi	<i>Pflp</i>	2.7 ± 1.2	1.7 ± 1.7	45.8 ± 23.2	66.7 ± 33.3	3
INU11212-15	Sukali ndiizi	<i>Pflp</i>	2.3 ± 1.4	0.0 ± 0.0	54.2 ± 29.2	100.0 ± 0.0	3
INU11212-19	Sukali ndiizi	<i>Pflp</i>	2.5 ± 2.5	0.0 ± 0.0	50.0 ± 50.0	100.0 ± 0.0	3
INU11212-24	Sukali ndiizi	<i>Pflp</i>	3.3 ± 1.7	1.7 ± 1.7	33.3 ± 33.3	66.7 ± 33.3	3
INU11211-13	Sukali ndiizi	<i>Hrap</i>	2.7 ± 1.2	3.3 ± 1.7	50.0 ± 28.87	33.3 ± 33.3	4
INU11211-28	Sukali ndiizi	<i>Hrap</i>	2.3 ± 1.4	3.3 ± 1.7	55.6 ± 29.4	33.3 ± 33.3	4
INU11211-36	Sukali ndiizi	<i>Hrap</i>	2.3 ± 1.4	3.3 ± 1.7	56.7 ± 29.6	33.3 ± 33.3	4
INU11211-40	Sukali ndiizi	<i>Hrap</i>	3.3 ± 1.7	3.3 ± 1.7	33.3 ± 33.3	33.3 ± 33.3	4
INU11212-02	Sukali ndiizi	<i>Pflp</i>	2.0 ± 1.5	3.3 ± 1.7	58.3 ± 30.0	33.3 ± 33.3	4
INU11212-04	Sukali ndiizi	<i>Pflp</i>	2.7 ± 1.4	3.3 ± 1.7	44.4 ± 29.4	33.3 ± 33.3	4
Nakinyika nontransgenic control	Nakinyika	-	4.0 ± 0.6	5.0 ± 0.0	14.3 ± 8.2	0.0 ± 0.0	4
Sukali ndiizi nontransgenic control	Sukali ndiizi	-	5.0 ± 0.0	5.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	4

*Mean of three replicates of each line.

Disease severity (score): 0- no symptoms, 1- only one leaf wilted, 2- 2 to 3 leaves wilted, 3- 4 to 5 leaves wilted, 4- all the leaves wilted but plant still alive, 5- whole plant died.

Resistance (%) = (Reduction in wilting of transgenic line / Mean wilting in nontransgenic control) X 100

Supplementary Table 2: Agronomic performance of transgenic lines in comparison to control nontransgenic plants.

Transgenic Line	No. of functional leaves Mean $\pm$ S.E.	Plant height at flowering (cm) Mean $\pm$ S.E.	No. of days to flowering Mean $\pm$ S.E.	Pseudostem girth (cm) Mean $\pm$ S.E.	Bunch weight (kg) Mean $\pm$ S.E.	No. of hands Mean $\pm$ S.E.	No. of fruits Mean $\pm$ S.E.	Weight of each fruit (g) Mean $\pm$ S.E.
Sukali ndiizi nontransgenic control	11.6 $\pm$ 0.2	278.8 $\pm$ 3.9	311.4 $\pm$ 3.9	42.2 $\pm$ 0.7	8.2 $\pm$ 0.3	8.5 $\pm$ 0.2	110.9 $\pm$ 3.7	59.0 $\pm$ 1.7
INU11211-02	12.0 $\pm$ 0.0	296.7 $\pm$ 8.8 ^G	298.0 $\pm$ 4.0	43.0 $\pm$ 1.0	8.5 $\pm$ 1.0	9.3 $\pm$ 0.3	128.3 $\pm$ 11.4	57.4 $\pm$ 10.5
INU11211-03	11.3 $\pm$ 0.3	296.7 $\pm$ 8.8 ^G	303.7 $\pm$ 2.7	46.0 $\pm$ 0.6 ^G	10.0 $\pm$ 0.5	10.0 $\pm$ 0.6 ^G	156.0 $\pm$ 14.1 ^G	48.5 $\pm$ 5.0 ^L
INU11211-04	11.7 $\pm$ 0.3	291.7 $\pm$ 4.4	309.0 $\pm$ 3.5	43.7 $\pm$ 1.2	8.5 $\pm$ 1.0	9.7 $\pm$ 0.3 ^G	156.3 $\pm$ 10.1 ^G	53.6 $\pm$ 8.1
INU11211-05	10.0 $\pm$ 1.2 ^L	240 $\pm$ 10.4 ^L	338.7 $\pm$ 19.2 ^G	43.0 $\pm$ 2.0	4.7 $\pm$ 0.3 ^L	4.7 $\pm$ 0.3 ^L	61.3 $\pm$ 6.4 ^L	47.7 $\pm$ 12.7 ^L
INU11211-06	11.3 $\pm$ 0.7	303.3 $\pm$ 9.3 ^G	278.3 $\pm$ 4.6 ^L	42.3 $\pm$ 0.7	8.8 $\pm$ 0.3	9.7 $\pm$ 0.3 ^G	129.7 $\pm$ 1.5	65.9 $\pm$ 7.2
INU11211-07	12.3 $\pm$ 0.3	290.0 $\pm$ 7.6	309.7 $\pm$ 16.7	43.3 $\pm$ 1.8	9.0 $\pm$ 0.6	9.7 $\pm$ 0.7 G	115.7 $\pm$ 5.3	60.5 $\pm$ 6.8
INU11211-08	11.7 $\pm$ 0.7	286.7 $\pm$ 3.3	314.7 $\pm$ 11.6	44.0 $\pm$ 1.7	8.3 $\pm$ 1.3	9.7 $\pm$ 0.3 G	125.7 $\pm$ 10.7	58.2 $\pm$ 5.3
INU11211-10	9.3 $\pm$ 0.3 ^L	273.3 $\pm$ 4.4	300.3 $\pm$ 2.3	38.7 $\pm$ 0.7 ^L	7.0 $\pm$ 0.0	8.7 $\pm$ 0.9	106.0 $\pm$ 2.1	55.0 $\pm$ 3.5
INU11211-11	11.3 $\pm$ 0.3	280.0 $\pm$ 5.0	271.0 $\pm$ 2.0 ^L	40.7 $\pm$ 0.3	9.0 $\pm$ 0.6	9.7 $\pm$ 0.3 ^G	121.0 $\pm$ 4.0	64.2 $\pm$ 3.9
INU11211-13	11.7 $\pm$ 0.3	291.7 $\pm$ 6.0	318.3 $\pm$ 7.0	41.7 $\pm$ 0.3	8.7 $\pm$ 0.3	9.3 $\pm$ 0.3	122.3 $\pm$ 5.7	53.4 $\pm$ 4.6
INU11211-14	11.0 $\pm$ 0.6	280.0 $\pm$ 5.0	328.7 $\pm$ 8.4	37.7 $\pm$ 3.0 ^L	5.3 $\pm$ 0.7 ^L	8.7 $\pm$ 0.9	95.3 $\pm$ 16.5	49.1 $\pm$ 1.2 ^L
INU11211-15	12.0 $\pm$ 0.0	290.0 $\pm$ 0.0	310.6 $\pm$ 14.6	40.8 $\pm$ 2.6	8.9 $\pm$ 1.8	9.0 $\pm$ 0.0	110.5 $\pm$ 17.7	67.6 $\pm$ 0.0
INU11211-16	11.7 $\pm$ 0.3	291.7 $\pm$ 6.0	311.0 $\pm$ 4.6	43.0 $\pm$ 1.0	8.0 $\pm$ 0.8	9.0 $\pm$ 0.6	133.7 $\pm$ 2.7 ^G	50.3 $\pm$ 6.6
INU11211-17	12.7 $\pm$ 0.3 ^G	288.3 $\pm$ 8.3	303.0 $\pm$ 9.6 ^L	42.0 $\pm$ 1.0	8.5 $\pm$ 0.8	8.7 $\pm$ 0.3	106.0 $\pm$ 10.0	59.7 $\pm$ 3.5
INU11211-18	12.7 $\pm$ 0.3 ^G	293.3 $\pm$ 6.7	286.0 $\pm$ 3.1	42.7 $\pm$ 0.9	10.3 $\pm$ 0.7 ^G	9.7 $\pm$ 0.3 ^G	128.3 $\pm$ 4.5	65.4 $\pm$ 5.3
INU11211-19	12.0 $\pm$ 0.0	286.7 $\pm$ 1.7	312.3 $\pm$ 7.0	42.0 $\pm$ 1.7	7.8 $\pm$ 0.4	8.7 $\pm$ 0.3	116.7 $\pm$ 8.4	57.1 $\pm$ 3.1
INU11211-21	11.7 $\pm$ 0.3	290.0 $\pm$ 5.8	319.7 $\pm$ 3.9	44.0 $\pm$ 0.6	9.0 $\pm$ 1.0	8.7 $\pm$ 0.3	130.7 $\pm$ 5.8	52.7 $\pm$ 1.0
INU11211-22	11.3 $\pm$ 0.3	245.0 $\pm$ 10.0 ^L	337.3 $\pm$ 10.1 ^G	42.7 $\pm$ 1.8	5.0 $\pm$ 0.6 ^L	6.3 $\pm$ 1.5 ^L	81.3 $\pm$ 16.7 ^L	44.2 $\pm$ 5.4 ^L
INU11211-24	12.0 $\pm$ 1.2	296.7 $\pm$ 4.4 ^G	301.3 $\pm$ 9.1	43.7 $\pm$ 0.9	9.3 $\pm$ 0.9	9.0 $\pm$ 0.0	126.0 $\pm$ 5.9	55.9 $\pm$ 1.5
INU11211-25	11.0 $\pm$ 0.0	271.7 $\pm$ 26.2	304.7 $\pm$ 1.7	43.0 $\pm$ 0.6	9.3 $\pm$ 0.9	9.0 $\pm$ 0.6	122.7 $\pm$ 9.0	60.8 $\pm$ 2.4
INU11211-26	11.7 $\pm$ 0.7	286.7 $\pm$ 4.4	297.3 $\pm$ 7.4	40.7 $\pm$ 0.3	8.0 $\pm$ 0.6	10.0 $\pm$ 0.6 ^G	111.3 $\pm$ 11.5	54.2 $\pm$ 4.5
INU11211-27	11.7 $\pm$ 0.3	295.0 $\pm$ 12.6	318.3 $\pm$ 6.1	42.7 $\pm$ 1.2	10.3 $\pm$ 0.9 ^G	9.7 $\pm$ 0.3 ^G	128.7 $\pm$ 6.4	59.7 $\pm$ 4.3
INU11211-28	11.0 $\pm$ 0.0	291.7 $\pm$ 4.4	317.0 $\pm$ 4.0	43.3 $\pm$ 1.2	11.3 $\pm$ 0.3 ^G	10.0 $\pm$ 0.0 ^G	129.7 $\pm$ 2.3	56.9 $\pm$ 4.5
INU11211-29	11.7 $\pm$ 0.3	301.7 $\pm$ 10.1 ^G	333.0 $\pm$ 7.0 ^G	45.3 $\pm$ 1.2 ^G	9.7 $\pm$ 0.9	11.0 $\pm$ 1.0 ^G	149.0 $\pm$ 15.5 ^G	57.5 $\pm$ 2.9
INU11211-30	11.7 $\pm$ 0.3	303.3 $\pm$ 3.3 ^G	334.0 $\pm$ 7.8 ^G	45.3 $\pm$ 1.5 ^G	11.7 $\pm$ 0.9 ^G	11.0 $\pm$ 1.0 ^G	152.7 $\pm$ 9.9 ^G	56.3 $\pm$ 2.5
INU11211-31	12.0 $\pm$ 0.0	285.0 $\pm$ 2.9	330.0 $\pm$ 5.2 G	44.3 $\pm$ 1.5	9.0 $\pm$ 0.6	10.0 $\pm$ 0.6 ^G	135.3 $\pm$ 4.1 ^G	56.8 $\pm$ 0.4
INU11211-32	12.0 $\pm$ 0.0	273.3 $\pm$ 10.1	325.0 $\pm$ 8.5	42.3 $\pm$ 0.9	7.0 $\pm$ 1.2	9.7 $\pm$ 1.2 G	135.3 $\pm$ 18.3 ^G	43.3 $\pm$ 2.9 ^L
INU11211-33	12.0 $\pm$ 0.0	290.0 $\pm$ 5.0	313.0 $\pm$ 11.3	43.0 $\pm$ 0.6	9.7 $\pm$ 0.9	9.3 $\pm$ 0.3	130.7 $\pm$ 0.9	61.8 $\pm$ 2.2
INU11211-34	12.5 $\pm$ 0.5	312.5 $\pm$ 27.5 ^G	350.5 $\pm$ 7.5 ^G	44.0 $\pm$ 1.0	7.0 $\pm$ 0.0	10.5 $\pm$ 1.5 ^G	148.5 $\pm$ 6.5 ^G	36.2 $\pm$ 1.0 ^L
INU11211-35	12.3 $\pm$ 0.3	290.0 $\pm$ 2.9	315.7 $\pm$ 4.7	44.3 $\pm$ 0.7	10.3 $\pm$ 0.7 ^G	9.3 $\pm$ 1.2	131 $\pm$ 13.4 ^G	56.1 $\pm$ 5.1
INU11211-36	12.7 $\pm$ 0.3 ^G	286.7 $\pm$ 3.3	327.0 $\pm$ 9.3	44.7 $\pm$ 0.9	9.3 $\pm$ 1.2	8.7 $\pm$ 0.3	121.3 $\pm$ 8.8	54.7 $\pm$ 2.1
INU11211-37	11.7 $\pm$ 0.3	303.3 $\pm$ 7.3 ^G	326.7 $\pm$ 7.8	45.7 $\pm$ 0.3 G	10.3 $\pm$ 0.9 ^G	10.3 $\pm$ 0.3 ^G	122.0 $\pm$ 9.7	61.4 $\pm$ 4.4
INU11211-38	12.7 $\pm$ 0.3 ^G	301.7 $\pm$ 7.3 ^G	325.7 $\pm$ 11.7	44.7 $\pm$ 0.7	7.3 $\pm$ 1.5	9.3 $\pm$ 0.7	132.7 $\pm$ 9.8 ^G	59.7 $\pm$ 10.3
INU11211-39	11.3 $\pm$ 0.3	296.7 $\pm$ 8.3 ^G	319.7 $\pm$ 3.8	44.0 $\pm$ 2.1	7.7 $\pm$ 1.3	8.3 $\pm$ 0.3	120.3 $\pm$ 9.8	51.8 $\pm$ 3.3
INU11211-40	12.3 $\pm$ 0.7	290.0 $\pm$ 5.8	330.7 $\pm$ 6.5 ^G	46.3 $\pm$ 0.7 ^G	7.7 $\pm$ 0.9	9.3 $\pm$ 0.7	127.7 $\pm$ 1.3	53.0 $\pm$ 2.3
INU11212-01	12.0 $\pm$ 0.6	288.3 $\pm$ 7.3	290.3 $\pm$ 10.3 ^L	42.3 $\pm$ 0.7	8.5 $\pm$ 0.5	9.3 $\pm$ 0.3	125.3 $\pm$ 10.9	58.3 $\pm$ 5.7

INU11212-02	11.0 ± 0.0	285.0 ± 8.7	310.3 ± 4.9	43.7 ± 1.5	8.7 ± 2.2	8.7 ± 0.7	112.3 ± 10.8	55.0 ± 2.3
INU11212-03	11.7 ± 0.3	286.7 ± 4.4	323.7 ± 2.2	45.3 ± 0.7 G	8.7 ± 0.9	9.0 ± 0.6	132.3 ± 15.8 ^G	59.0 ± 1.2
INU11212-04	11.3 ± 0.3	287.5 ± 2.5	310.3 ± 7.3	44.5 ± 0.5	9.5 ± 0.5	10.0 ± 0.0 ^G	137.5 ± 3.5 ^G	62.6 ± 5.2
INU11212-05	11.7 ± 0.3	290.0 ± 7.6	304.7 ± 9.7	42.7 ± 1.2	10.0 ± 0.6	9.3 ± 0.3	126.3 ± 5.8	66.1 ± 3.5
INU11212-06	11.3 ± 0.3	288.3 ± 7.3	311.7 ± 4.5	42.7 ± 0.9	10.0 ± 1.2	9.3 ± 0.3	132.0 ± 8.5 ^G	61.7 ± 4.8
INU11212-07	11.7 ± 0.3	303.3 ± 1.7 ^G	308.0 ± 8.6	39.0 ± 3.0 ^L	9.0 ± 0.6	9.7 ± 0.3 ^G	133.0 ± 0.6 ^G	60.0 ± 2.5
INU11212-08	11.0 ± 0.6	296.7 ± 11.7 ^G	292.3 ± 1.9 ^L	43.0 ± 2.0	9.7 ± 1.5	10.7 ± 0.7 ^G	135.7 ± 17.6 ^G	60.8 ± 3.4
INU11212-09	12.3 ± 0.3	308.3 ± 4.4 ^G	307.7 ± 9.2	45.7 ± 0.9 ^G	10.0 ± 0.0	10.3 ± 0.3 ^G	127.7 ± 5.0	64.4 ± 7.9
INU11212-10	11.0 ± 0.6	276.7 ± 8.8	319.3 ± 2.9	43.0 ± 2.6	8.0 ± 1.5	8.3 ± 0.3	110.0 ± 10.6	58.1 ± 3.5
INU11212-11	12.0 ± 0.6	288.3 ± 7.3	300.0 ± 6.7	42.0 ± 2.1	8.7 ± 1.3	9.0 ± 0.6	124.0 ± 8.7	55.7 ± 4.6
INU11212-12	10.7 ± 0.3	306.7 ± 6.0 ^G	314.3 ± 9.2	45.7 ± 0.3 ^G	9.7 ± 1.5	9.3 ± 0.3	133.0 ± 4.7 ^G	56.9 ± 8.0
INU11212-13	12.7 ± 0.9 ^G	273.3 ± 14.8	335.7 ± 7.9 ^G	41.3 ± 3.2	7.7 ± 0.3	9.3 ± 0.3	120.7 ± 7.7	51.4 ± 1.2
INU11212-14	11.0 ± 0.6	283.3 ± 12.0	292.3 ± 11.5 ^L	42.7 ± 1.9	9.7 ± 0.7	9.3 ± 0.3	108.0 ± 13.0	62.8 ± 3.7
INU11212-15	12.7 ± 0.3 ^G	296.7 ± 6.7 ^G	317.3 ± 11.7	42.0 ± 1.5	9.7 ± 0.9	10.0 ± 0.0 ^G	143.3 ± 14.8 ^G	58.5 ± 8.8
INU11212-16	11.3 ± 0.3	280.0 ± 5.8	319.7 ± 4.1	39.0 ± 3.5 ^L	7.0 ± 0.6	8.3 ± 0.9	99.7 ± 8.5	52.2 ± 3.3
INU11212-17	10.3 ± 0.3 ^L	265.0 ± 7.6	300.0 ± 11.3	40.0 ± 1.2	5.2 ± 0.9 ^L	8.0 ± 0.6	99.3 ± 8.2	39.1 ± 2.1 ^L
INU11212-19	11.5 ± 0.5	277.5 ± 2.5	343.5 ± 24.5 ^G	40.0 ± 1.0	8.0 ± 1.0	9.5 ± 0.5	115.5 ± 19.5	52.7 ± 0.1
INU11212-20	12.0 ± 0.6	298.3 ± 4.4 ^G	304.3 ± 10.3	43.0 ± 2.1	9.7 ± 1.3	9.7 ± 0.3 ^G	123.0 ± 9.0	62.6 ± 6.6
INU11212-21	11.3 ± 0.7	316.7 ± 6.7 ^G	345.3 ± 5.9 ^G	45.0 ± 1.2	12.7 ± 0.7 ^G	11.3 ± 0.7 ^G	165.7 ± 2.0 ^G	63.6 ± 4.0
INU11212-22	13.0 ± 0.6 ^G	293.3 ± 1.7	323.3 ± 13.5	43.7 ± 1.3	10.0 ± 1.0	9.0 ± 0.0	138.3 ± 6.6 ^G	60.3 ± 6.3
INU11212-23	12.3 ± 0.3	291.7 ± 6.0	331.0 ± 9.5 ^G	46.3 ± 1.9 ^G	9.0 ± 1.0	9.3 ± 0.3	133.0 ± 8.0 ^G	48.4 ± 3.0 ^L
INU11212-24	12.0 ± 0.6	303.3 ± 8.8 ^G	328.3 ± 6.9	44.3 ± 1.7	10.3 ± 0.9 ^G	10.3 ± 0.3 ^G	138.7 ± 9.9 ^G	58.1 ± 2.7
P-value	0.0003	< 0.0001	< 0.0001	0.0095	< 0.0001	< 0.0001	< 0.0001	0.0252
LSD (to compare line with control)	1.0	18.2	18.5	3.3	1.9	1.3	20.9	10.9
LSD (to compare any two lines)	1.3	23.2	23.6	4.2	2.5	1.6	26.7	23.9
Transgenic Line	No. of functional leaves Mean* ± S.E	Plant height at flowering (cm) Mean* ± S.E	No. of days to flowering Mean* ± S.E	Pseudostem girth (cm) Mean* ± S.E	Bunch weight (kg) Mean* ± S.E	No. of hands Mean* ± S.E	No. of fruits Mean* ± S.E	Weight of each fruit (g) Mean* ± S.E
Nakinyika nontransgenic control	9.7 ± 0.2	285.5 ± 3.9	354.7 ± 5.5	44.6 ± 1.2	12.4 ± 0.9	8.8 ± 0.4	116.4 ± 7.5	84.8 ± 3.6
INU12212-02	9.7 ± 1.7	261.7 ± 3.3 ^L	327.3 ± 13.6 ^L	41.3 ± 0.7	10.0 ± 1.0	6.7 ± 0.3 ^L	81.3 ± 11.8 ^L	85.3 ± 17.7
P-value	0.9713	0.009	0.0487	0.1874	0.2306	0.0159	0.043	0.9641
LSD (to compare line with control)	1.8	15.1	24.7	4.6	3.7	1.5	30.7	21.9

*Mean of three replicates of each line. Mean of transgenic line with letter 'G' are significantly greater than mean of nontransgenic control plants; and mean of line with letter 'L' are significantly less than mean of nontransgenic control plants (at P = 0.05).