

Respond of GM sugarcane to drought stress

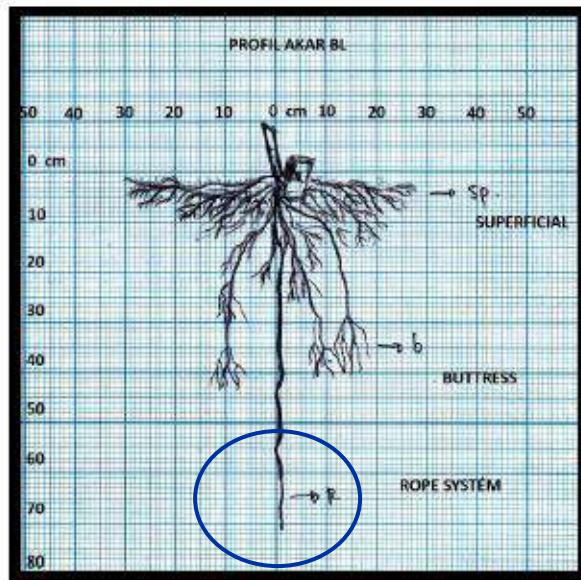
<i>Event</i>	Lenght of water stress (days)		
	Wilting	Permanent Wilting	Dried
NXI-1T	13	> 30	> 30
NXI-2T	13	> 30	> 30
CF1426-NT	8	12	13
NXI-3T	12	> 30	> 30
NXI-4T	12	> 30	> 30
NXI-5T	14	> 30	> 30
BL579-NT	8	28	29
NXI-6T	13	> 30	> 30
JT26-NT	9	11	13

Drought stress was treated to sugarcane by stop watering

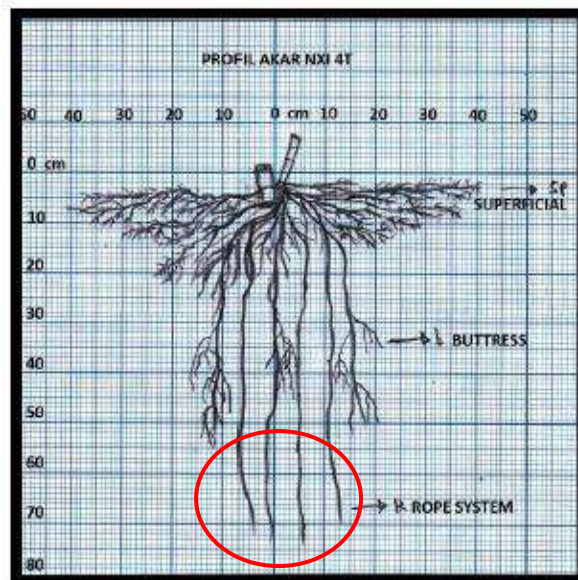
Source : PT. Perkebunan Nusantara XI



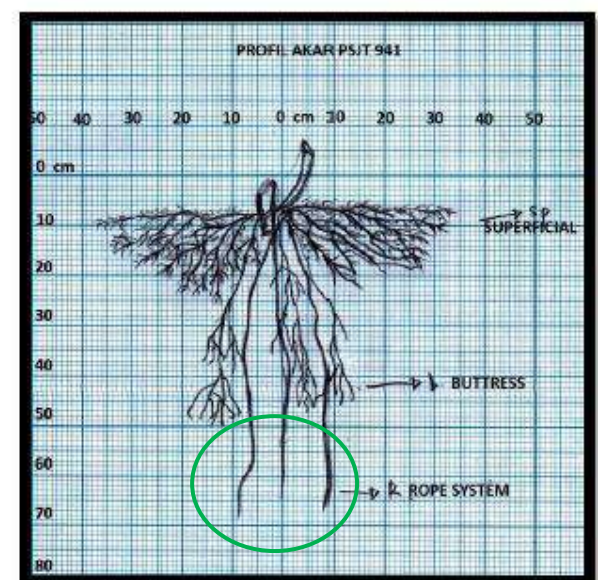
Root profile diagram after drought stress



BL



NXI-4T



PSJT 941

Source: *Laboratorium Bioteknologi PTPN XI*

Productivity and betaine content of drought tolerant GM sugarcane on rain fed field

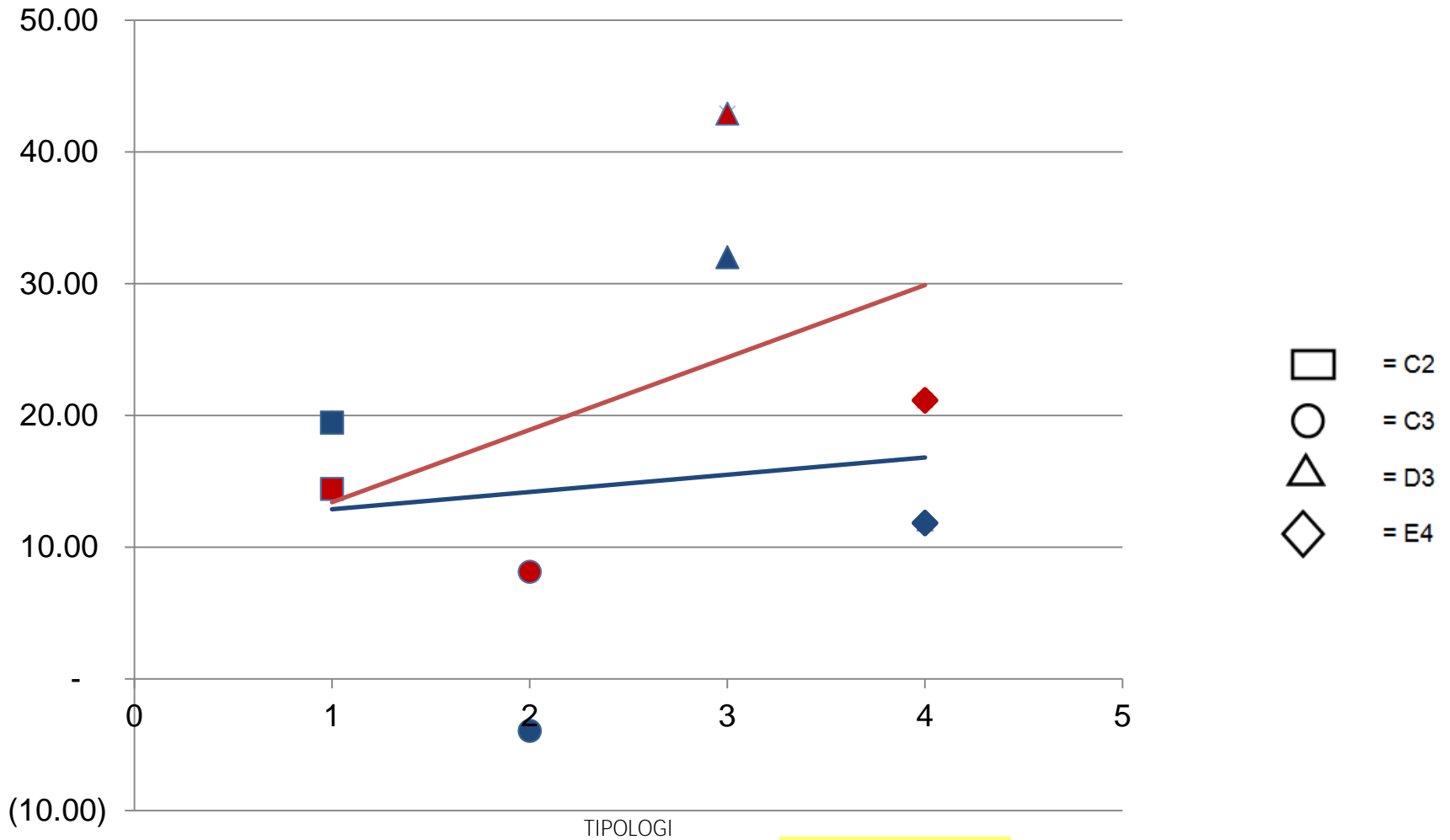
Event	Weight/ Ha (kui)	Rend. (%)	Crystal/ Ha (kui)	NMG thd BL	Betaine (ppm)
NXI - 1T	585	8,31	48,76	120,10	567,58
NXI - 2T	555	8,67	48,26	118,87	356,50
NXI - 3T	505	8,33	42,19	103,92	182,41
NXI - 4T	540	8,47	45,88	113,00	457,37
NXI - 5T	504	8,51	43,02	105,96	880,28
NXI - 6T	543	8,49	46,24	113,89	526,71
BL-579- NT	517	7,83	40,60	100,00	not detected

Source : PT Perkebunan Nusantara XI

PRODUCTIVITY IMPROVEMENT (%) COMPARED TO WILD TYPE at Different Climate Type

Climate Type	Biomass	Sugar content
C2	19.46	14.42
C3	-3.95	8.13
D3	32.02	42.91
E4	11.83	21.15

DROUGHT TOLERANCE SUGARCANE PRODUCTIVITY At Different Climate Type



Red : Sugar

Blue : Biomass

Summary of Potential Productivity of GM Sugarcane NXI 4T in multi location field test

Plant Cane

- Cane (T/Ha) : 911 ± 355.08
- Rendement (%) : 8.45 ± 1.44
- Sugar (T/Ha) : 77.67 ± 38.13

Ratoon

- Cane (T/Ha) : 756.25 ± 246.15
- Rendement (%) : 8.08 ± 1.75
- Sugar (T/Ha) : 58.74 ± 12.61

PLANTING LOCATION

- suitable to be planted in rain fed land, especially at early rainy season
- NXI 4T is suitable to be planted in dry land on entisol and inceptisol soil, with type of climate E4 and D3

Study of Non Target Organism (NTO) – Insect Abundant

- Biology function of insect in Confined Field Trial stil well maintained.
- There's no GM sugarcane influence to insect abundant
- The different level between insect group caused by different type of environment



Pitfall trap

Water pan trap



Store in formalin solution

Study of invasiveness : Analyze of Invasiveness toward Weeds



The competition ability of GM sugarcane is lower than weeds (purslane and grass)

Dry weight of GM sugarcane declined more than two kinds of weed with method of replacement series

GM sugarcane is not invasive

Gene flow study

- LFT lay out design follow guidance
- Different period of planting with adjacent field
- Distance isolation to avoid spreading of voluntary seed
- Comparative study on the biology of sugarcane flower :
 - seeds are very small, low viability are difficult to germinate, do not have the dormancy, and cannot be grown on land naturally
- different time of flower maturity, sugarcane is a hibrid so non flowering/rare

LABORATORY TESTING

Food Safety Assessment

Genetic Stability

Substance Equivalence

Acute Toxicity

Allergenicity

Digestive Tract



- Comparative study Wild Type and Reference

- Animal testing using mice

- Homology / Similarity (software program)
- Allergene Reaction (animal testing using rat, IgE)

- Gastric enzyme
- Intestinal enzyme
- Protein analysis (SDS PAGE)

Allergenicity

Tabel 1. Rerata kadar IgE tikus (ng/ml) sesuai kelompok perlakuan

Kode sampel	Kadar IgE hari ke-0	Kadar IgE hari ke-7	% Peningkatan dr hari ke-0	T test 5%	Kadar IgE hr ke-14	% Peningkatan dr hari ke-0	T test 5%
KN	123.88	114.42	-7.64	s	100.34	-19.00	s
KP	88.88	120.88	36.00	s	93.11	4.76	n.s
P-4T	102.61	97.98	-4.51	s	83.42	-18.70	s
P-6T	94.03	97.48	3.67	n.s	88.96	-5.39	s
P-WT	99.95	94.62	-5.33	s	95.47	-4.48	n.s

Keterangan :

n.s = not significance = tidak berbeda nyata

s = significance = berbeda nyata

(Sumber data : Lab Farmakognosi dan Fitokimia, Fak. Farmasi Univ. Airlangga)

Allergenicity

Tabel 2. Program FASTA3 dari AllergenOnline Database dengan protein CDH dari *R. melliloti*

Sekuen GI	Organisme	Diskripsi	Panjang	E skor	% Identity	Aa alignment
78038796	<i>Malassezia sympodialis</i>	mala s 12 allergen precursor	618	4.4e-013	32.2	583
256429	<i>Glycine max</i>	Kunitz trypsin inhibitor	216	4.5	26.3	133
18770	<i>Glycine max</i>	Trypsin inhibitor type A	217	4.5	26.3	133
729764		Heat shock 70 kDa protein	643	6.4	29.5	61
83300369		Allergen Asp #	322	7.1	26.5	68
66847146	<i>Aspergillus fumigatus Af293</i>	Allergen Asp #	322	7.1	26.5	68
3005839	<i>Aspergillus fumigatus</i>	rAsp f4	286	7.6	26.5	68
1359600	<i>Castanea sativa</i>	Chitinase Ib	316	8.2	22.2	63

Persentase semua hasil alignmen dari full length protein CDH menunjukkan tingkat kemiripan dibawah 35% pada lebih dari 80 asam amino yang diuji dan nyata dibawah level minimum untuk dapat dikategorikan sebagai protein cross-reaktif (<50%).

Hasil analisa ini mengindikasikan bahwa tidak ada potensi alergen cross-reaksi pada protein CDH yang diekspresikan pada tanaman tebu toleran kekeringan.

Stability on digestive system

a. Heat stability

Tabel 1. Stabilitas pemanasan (heat stability) enzim CDH pada nira tebu PRG toleran kekeringan. Stabilitas enzim ditentukan dengan pemanasan nira tebu PRG pada suhu 37°C, 60°C, dan 100°C selama 30 menit.

Sample nira tebu PRG dan kontrol	Aktifitas enzim CDH (unit/gr berat basah) sesudah pemanasan		
	37°C	60°C	100°C
Event NXI-1T	62.93	53.13	0
Event NXI-4T	261.28	239.66	0
Event NXI-6T	984.96	956.02	0
Kontrol non-PRG	ND	ND	ND

Catatan : 1 unit enzim setara dengan 1 μmole H_2O_2 yang dihasilkan dari oksidasi 1 μmole choline menjadi betaine aldehyde per jam pada pH 7.5 suhu 37°C.

ND adalah *not detectable*

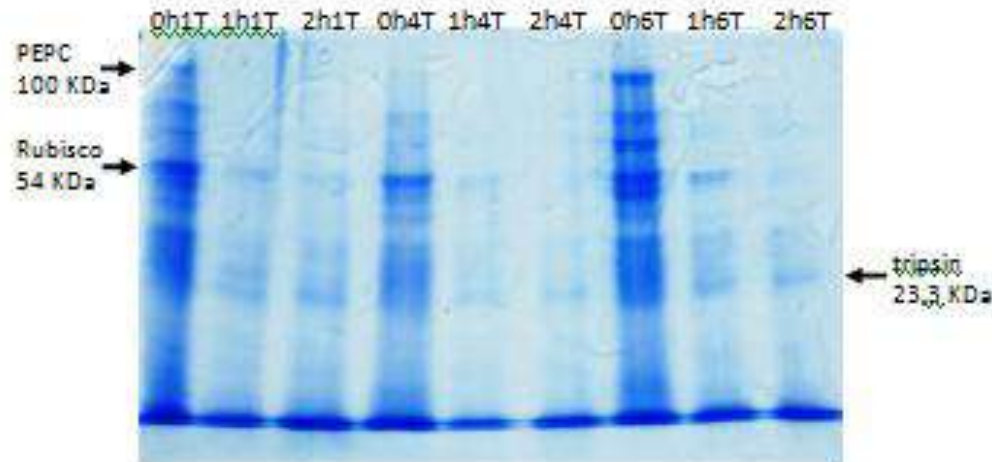
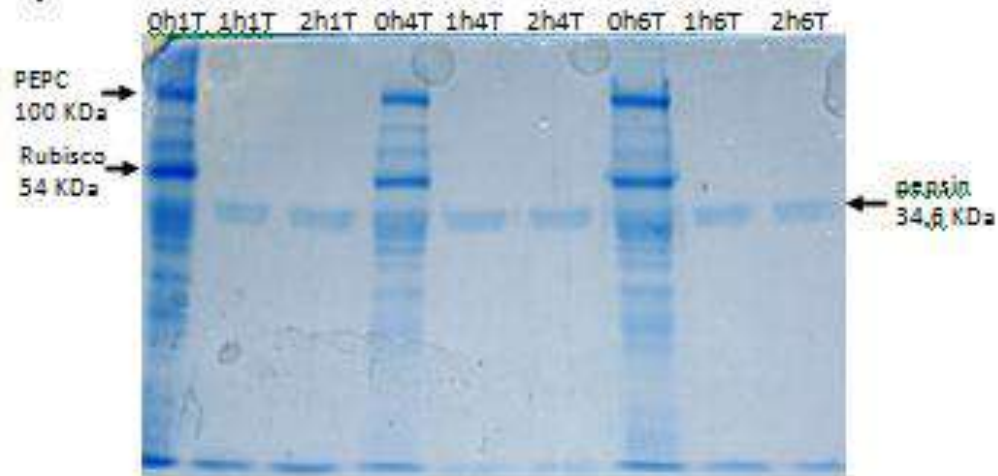
Stability on digestive system SGF (enzim pepsin) dan SIF (enzim tripsin)

Tabel 2. Stabilitas cerna (*digestion stability*) enzim CDH dalam nira tebu PRG toleran. Stabilitas cerna ditentukan dengan inkubasi enzim CDH dalam buffer SGF (pH 1.2) yang mengandung enzim pepsin atau dalam buffer SIF (pH 7.5) yang mengandung enzim tripsin.

Sample nira tebu PRG dan kontrol non-PRG	Aktifitas CDH sesudah inkubasi pepsin (unit/gr berat basah)			Aktifitas CDH sesudah inkubasi tripsin (unit/gr berat basah)		
	0 menit	30 menit	60 menit	0 menit	30 menit	60 menit
Event NXI-1T	0	0	0	13781.58	0	0
Event NXI-4T	0	0	0	941.51	0	0
Event NXI-6T	0	0	0	1292.21	0	0
Kontrol non-PRG	ND	ND	ND	ND	ND	ND

Catatan : 1unit enzim setara dengan 1 μmole H_2O_2 yang dihasilkan dari oksidasi 1 μmole choline menjadi betaine aldehyde per jam pada pH 7.5 suhu 37°C. ND adalah *not detectable*

Stability on digestive system Protein analysis



Gambar 3. Analisis SDS-PAGE protein daun tebu PRG sesudah inkubasi dengan enzim pepsin (atas) dan tripsin (bawah) selama 0, 60, dan 120 menit.

FIELD/LAB TESTING

Feed Safety Assessment

Genetic Stability

Substance Equivalence

Acute Toxicity

Protein digestion in ruminant

Homology with natural toxic protein

- Comparative study Wild Type and Reference

- Animal testing using mice

- Fermentation in rumen solutio
- Sugarcane shoots
- Protein digestibility value

- Homology / Similarity (software program)



Protein digestibility on ruminant

Hasil analisa dari sampel daun tebu BL dan NXI 4T sebagai berikut:

No	Varietas	Kandungan Zat Makanan		
		Bahan kering (%)	Abu * (%)	Protein kasar (%)
1	BL	21,25	8,84	5,77
2	NXI 4T	21,22	9,15	5,57

*). Berdasarkan 100% bahan kering.

Sumber. Lab. Nutrisi Fakultas Peternakan Universitas Brawijaya.

No	Varietas	Nilai Kecernaan Makanan		
		DCBK* (%)	DCBO* (%)	DCPK* (%)
1	BL	50,90	52,95	5,55
2	NXI 4T	49,91	51,50	2,87

*). Berdasarkan 100% bahan kering.

DCBK = derajat cerna bahan kering, DCBO = derajat cerna bahan organik, DCPK = Derajat cerna protein kasar

Sumber. Lab. Nutrisi Fakultas Peternakan Universitas Brawijaya.

RESUME

BIOSAFETY ASSESSMENT OF DROUGHT TOLERANCE SUGARCANE

Environment safety assessment :

No evidence of gene flow. No possibility of cross breeding between *Saccharum* species. Has no invasive or weedy character

Certificate no :

B-7945/MENLH/08/2011 – 26

Agustus 2011

Food safety assessment :

Sugar is a processing product
Proven non allergen and no toxic, digestable in mammals digestive system

Certificate no:

HK.04.1.52.10.12.6489 / 2012

– 9 Oktober 2012



RESUME

BIOSAFETY ASSESSMENT OF DROUGHT TOLERANCE SUGARCANE

Feed safety assessment :

Proven has the same quality of digestible protein in ruminant.
Has no similarity with naturally occur toxic protein

Certificate no :

214.2/Kpts/H/08/2018 – 20

Agustus 2018

PVT (Patent) :

20 years crop plant patent

Certificate no:

00379/PPVT/S/2016 – 23

Nopember 2016



DROUGHT TOLERANCE GM SUGARCANE AT PT PERKEBUNAN NUSANTARA XI

Officially released as commercial variety by Ministry of Agriculture (Kepmentan no. : 4571/Kpts/SR.120/8/2013 – 12 Agustus 2013)



ACKNOWLEDGEMENT :

Prof Bambang Sugiharto and team – CDAST, Unej

Dr Sony Suhandono and team, SITH, ITB.

Dr. Netty Ermawati and team, Politeknik Jember.

**Dr. Untung Murdiyatmo and Mr Harufumi Miwa –
PTPN XI and Ajico, Int**



THANK YOU