International Symposium on Cocoa Research

Transcriptomic response of cocoa (*Theobroma cacao* L.) genotypes to water-deficit stress: Implications for drought tolerance

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Outline

Cocoa in India

Screening for drought tolerance

Physiological and biochemical parameters for drought tolerance

Transcriptomics of cocoa genotypes to water-deficit stress

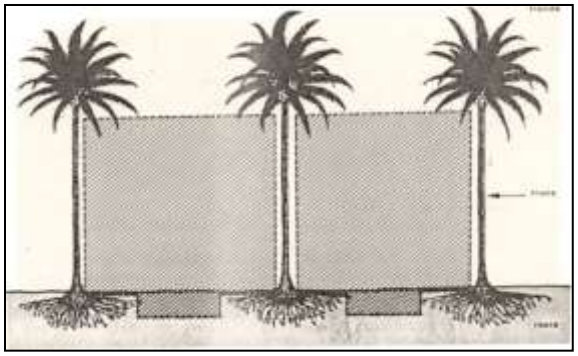
Conclusion

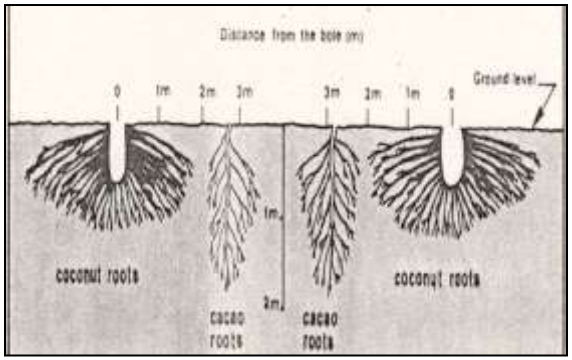
Cocoa in India

1798	Courtallam in Tirunelveli dt., 1873- Burliar fruit station
1930-1935	Plantings were done with Criollo and Forastero
1955	Research at Kallar/ Burliar under ICAR
	Western Ghat hills & plains- rains both monsoons, short dry seasons (Madras, Mysore and Malabar states)
1962	ICAR- Criollos in South India, Forasteros in NE India
1964	CPCRI (Arecanut + Cocoa, Coconut + Cocoa) Vittal, Peechi, Palode, Kahikuchi
1968-69	ICAR-CPCRI, Vittal- Improvement

(Malhotra et al., 2016)

Air/soil/root space in plantations





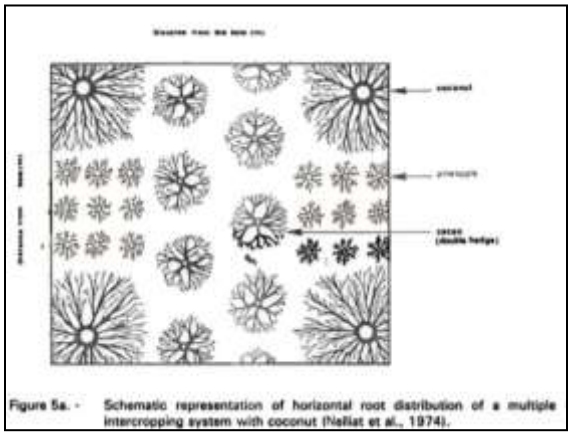
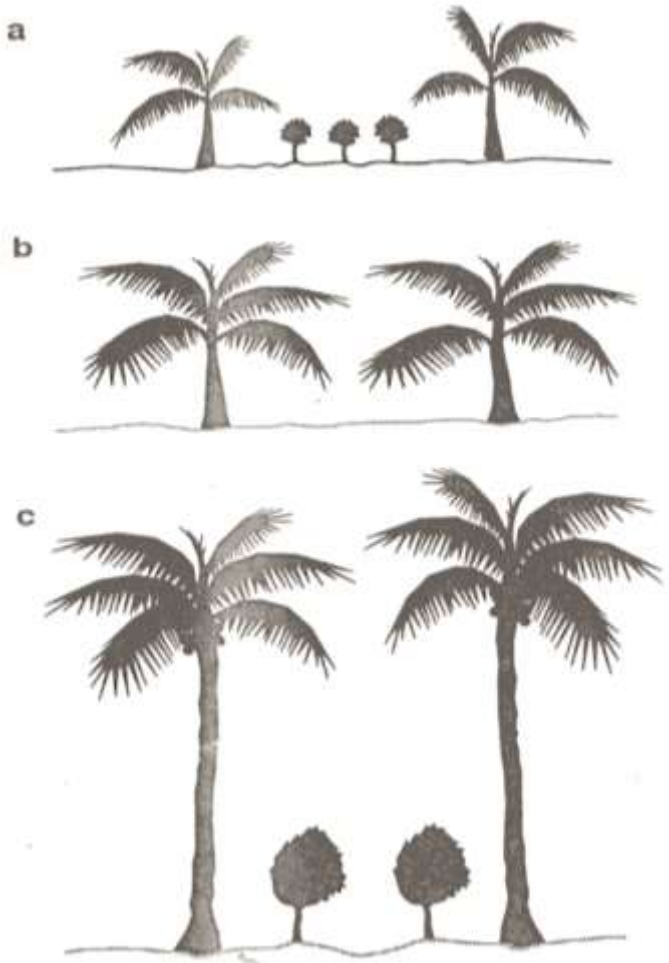


Figure 5a. - Schematic representation of horizontal root distribution of a multiple intercropping system with coconut (Nelliat et al., 1974).

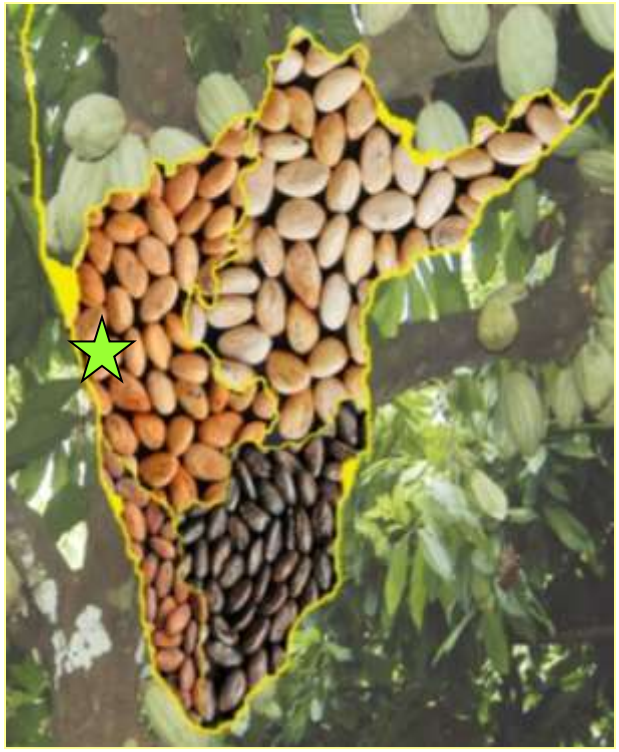


Nelliat et al ., World Crops 26(6): 262-266

Cocoa in Indian plantations

Cocoa in India -dry period of 4–6 months: Supplemental irrigation
Annual RF < 1200–1600 mm: significant losses in the development and productivity

Selection of drought tolerant genotypes at seedling stage : morpho-physiological traits to discriminate water deficit stress to accelerate breeding cycle



Cocoa in India

State	Area (000'ha)	Production (000' MT)	Productivity (kg/ha)
KER	17.36	10.10	850
KAR	14.21	03.90	525
AP	39.72	11.40	950
TN	32.08	02.90	350
Total	97.56	27.07	669

Demand of chocolate industry and confectionaries: 50,000 MT of dry bean per annum

(DCCD, GoI & © Statista 2022)

Screening for water-deficit stress tolerance

Alban et al., Ind J Plant Physiol. (2016) 21(1):23–30

Screening for water-deficit stress tolerance

- Stomatal resistance, chlorophyll fluorescence, water potential, specific leaf weight and epicuticular wax contents etc
- A total of 216 cocoa genotypes have so far been screened
- NC 23/43, NC 29/66 and NC 42/94 are drought tolerant and utilized in hybridization programmes
- 2 hybrids developed (VTLCH3 & VTLCH4)

(Apshara et al., 2019)

Screening for water-deficit stress tolerance

- thick leaf
- higher epicuticular wax content
- efficient stomatal closure, and
- high tissue elasticity

(Apshara et al., 2019; Balasimha et al ., 2013)

Screening for water-deficit stress tolerance

Soil moisture content (%)	20%-50% FC
Physiological traits	
Stomatal resistance (s/cm)	≥ 10.77
Conductance (mole/m^2/s)	≤ 0.36
Transpiration rate (m mol/m^2/s)	≤ 0.99
Leaf water potential (Bar)	≥ -19
Photo (µmol/m^2/s)	≥ 9.62
$CO_{2\ int}$ (ppm)	≥ 300
WUE (Pn/E)	≥ 10.49
Pn/C_{int}	≥ 0.032
Chlorophyll fluorescence (Fv/Fm)	≥ 0.31
Epicuticular wax (µg/cm^2)	≥ 20.58

Biochemical metabolites	
Total Soluble Sugar (µg/gMF)	≥ 16.86
Amino Acid (µg/gMF)	≥ 2.05
Proline (µg/gMF)	≥ 0.048
Protein (µgBSA/mgMF)	≥ 0.84

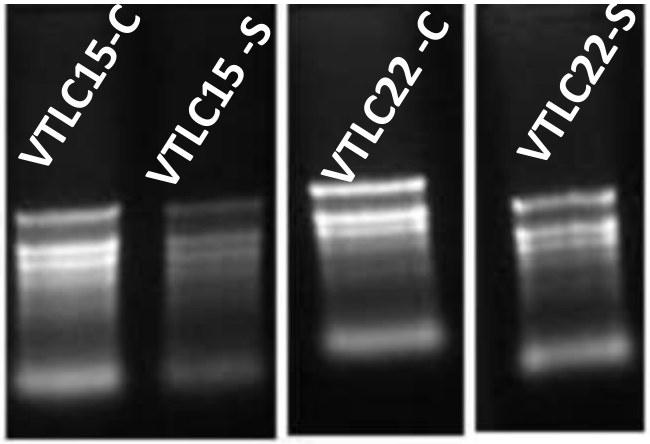
Antioxidant enzymes	
MDA (mmol)	≥ 0.04
SOD (Specific activity/ min/protein)	≥ 0.5
CAT (Specific activity/ min/protein)	≥ 0.07
POX (Specific activity/ min/protein)	≥ 0.83
PPO (Specific activity/ min/protein)	≥ 0.147

(Apshara et al., 2019; Balasimha 1999; Balasimha et al., 2013)

Water-deficit stress on the growth and physiology of cocoa seedlings

Physiological Parameters	VTLC22		VTLC15	
	Control (100% FC)	Stressed (50% FC)	Control (100% FC)	Stressed (50% FC)
Leaf water potential (bars)	-12.98	-16.20	-7.52	-12.03
Stomatal resistance (R_s)	2.51	11.47	3.04	7.61
Stomatal conductance (g_s) (mol m^{-2} s^{-1})	0.27	0.29	0.49	0.37
Water use efficiency (WUE) (Pn/E)	38.45	25.44	20.17	18.93

VTLC 22 : good photosynthetic activity; water use efficiency; high CO_2 accumulation
Good leaf gaseous exchange parameters



High quality read data statistics

Sample	No. of PE reads	No. of Total Reads	Data (Gb)
VTLC15 (Control)	34,851,626	69,703,252	10.3
VTLC15 (Stressed)	32,636,232	65,272,464	9.6
VTLC22 (Control)	28,976,082	57,952,164	8.5
VTLC22 (Stressed)	31,944,637	63,889,274	9.4

Analysis pipeline

High quality reads of cocoa transcriptome

T. Cacao (https://www.cacaogenomedb.org/Tcacao_genome_v1.1)

HQ reads aligned HISAT2 (version-hisat2-2.0.5)

SAMtools (version-0.1.18, http://samtools.sourceforge.net/)

Binary alignment/map (BAM) file

Mapped reads ratio (MRR) to the reference in each dataset

Differential gene expression (Cuffdiff in cufflinks package)

GO-BLAST2GO

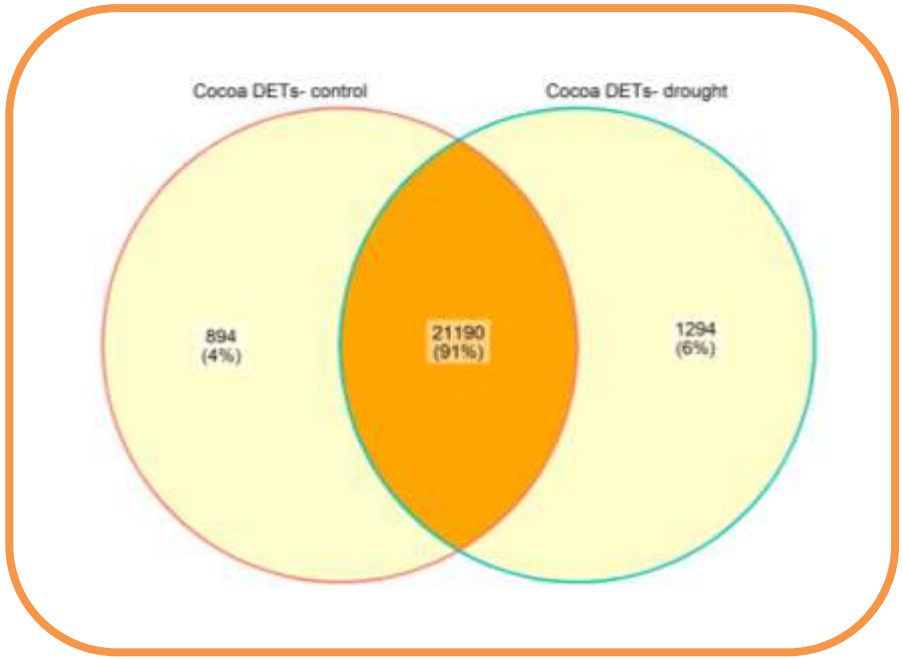
KEGG- KEGG automatic annotation server (KAAS)

lncRNA-INFERNAL v-1.1.1 tool

Statistics of mapped reads

Sample	% of mapped reads	No. of transcripts assembled
VTLC15 (Control)	92.98%	21995
VTLC15 (Stressed)	92.53%	22161
VTLC22 (Control)	94.46%	25572
VTLC22 (Stressed)	93.37%	22744

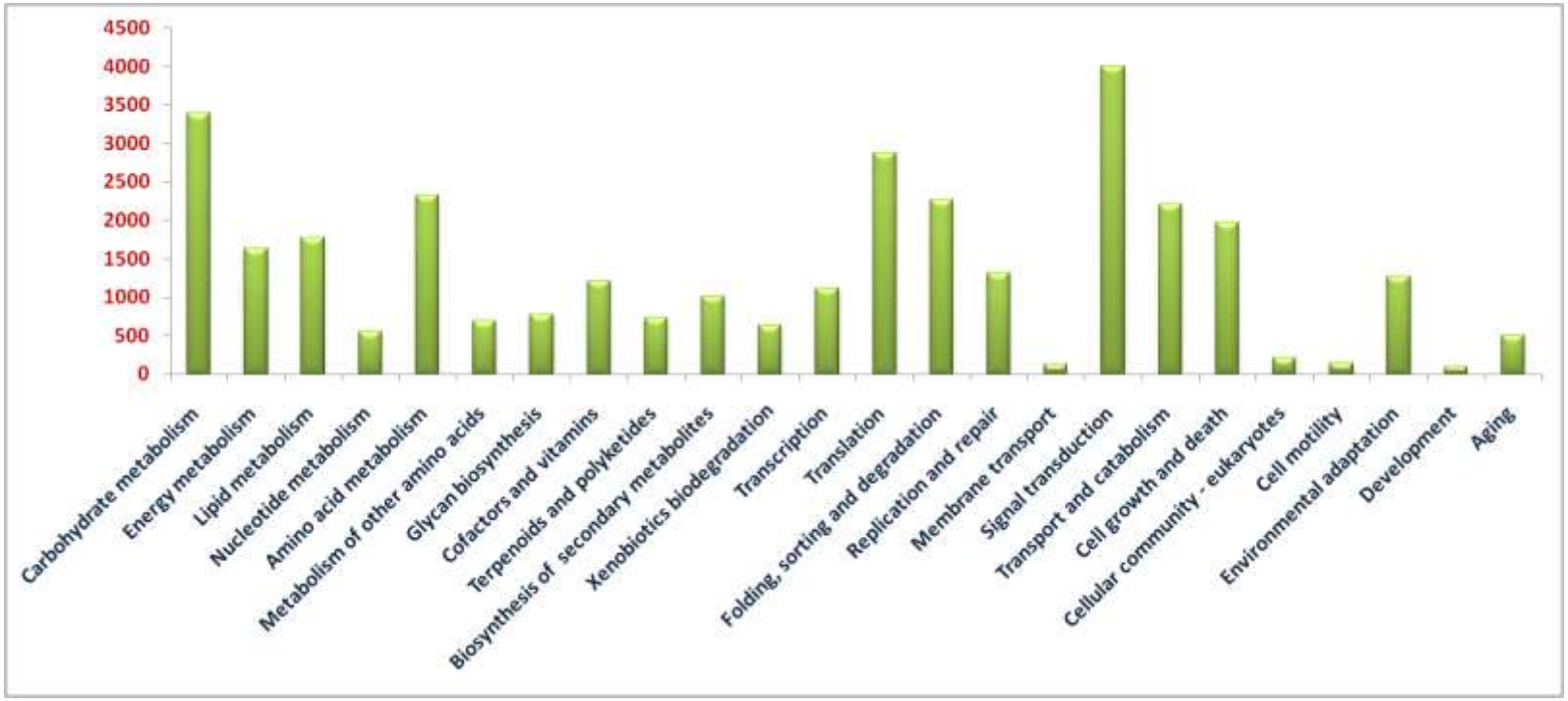
The clean RNA-seq (Illumina paired-end) reads mapped to the Cacao Genome Database (CGD) based on Matina 1-6 cultivar

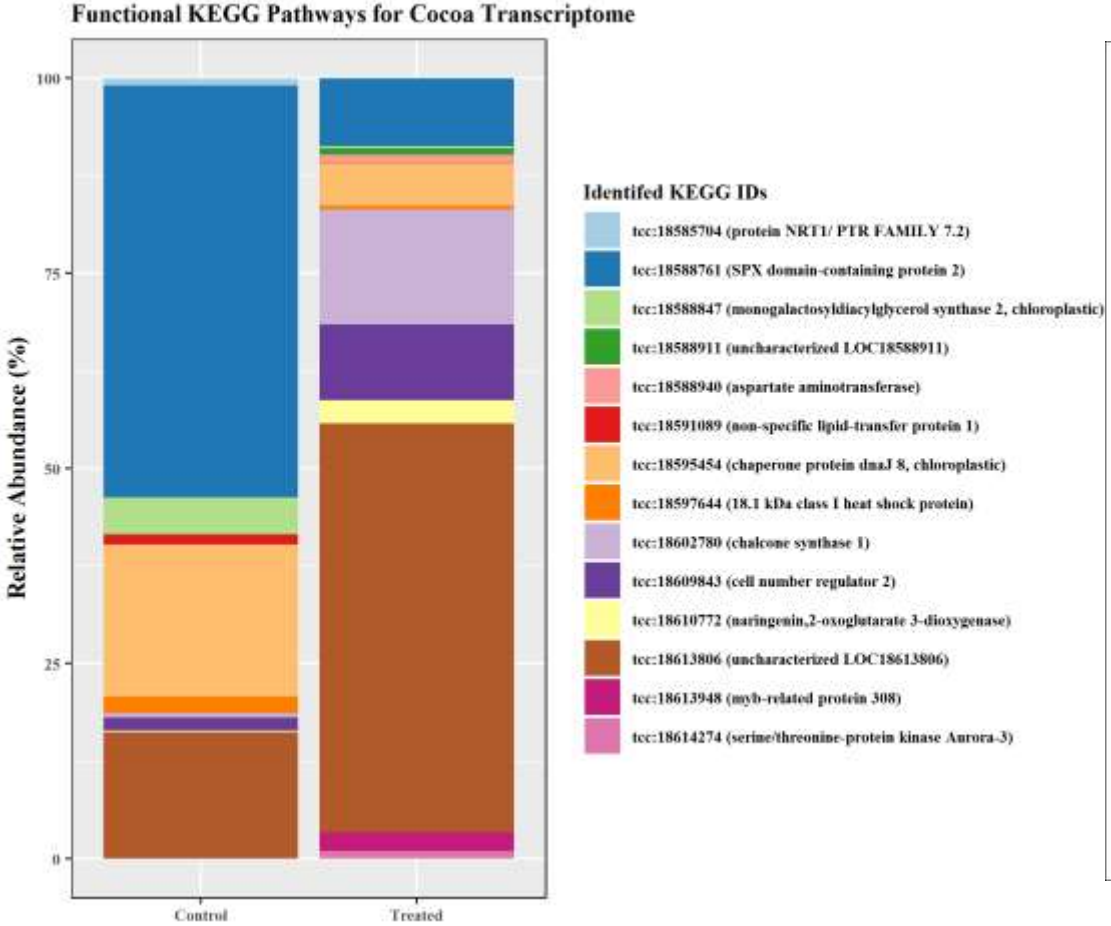


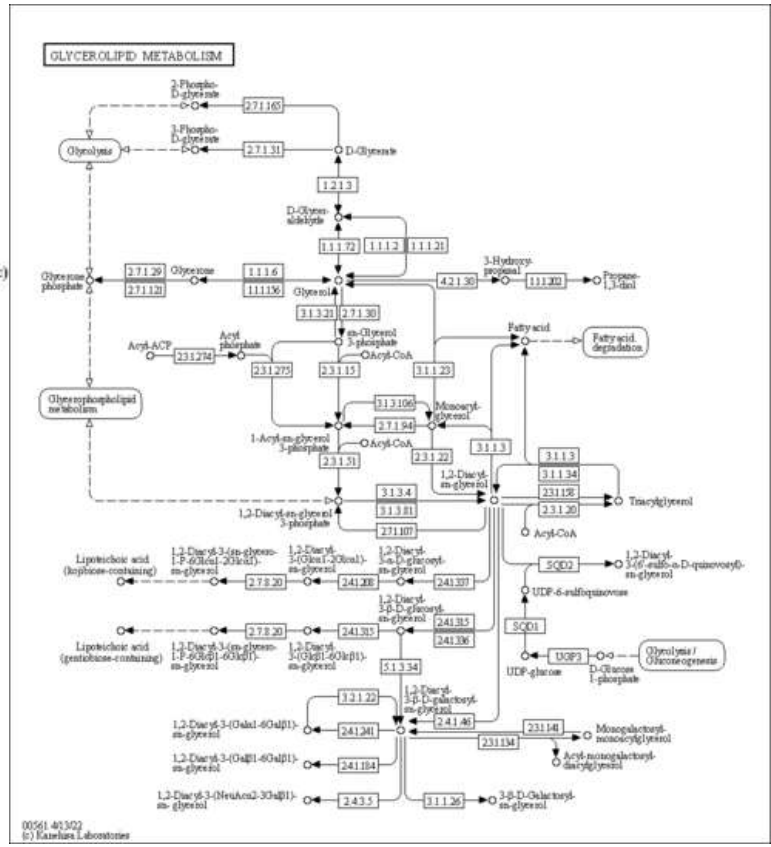
The clean RNA-seq (Illumina paired-end) reads mapped to the Cacao Genome Database (CGD) based on Matina 1-6 cultivar

Around 6% of the transcripts (1294) are modulated by drought in both the genotypes

Functional analysis of modulated transcripts-KEGG pathways







Drought in cocoa activates glycerolipid metabolism, chalcone synthase, genes involved in cell number regulator, aspartate amino transferases, MYB-related protein among others

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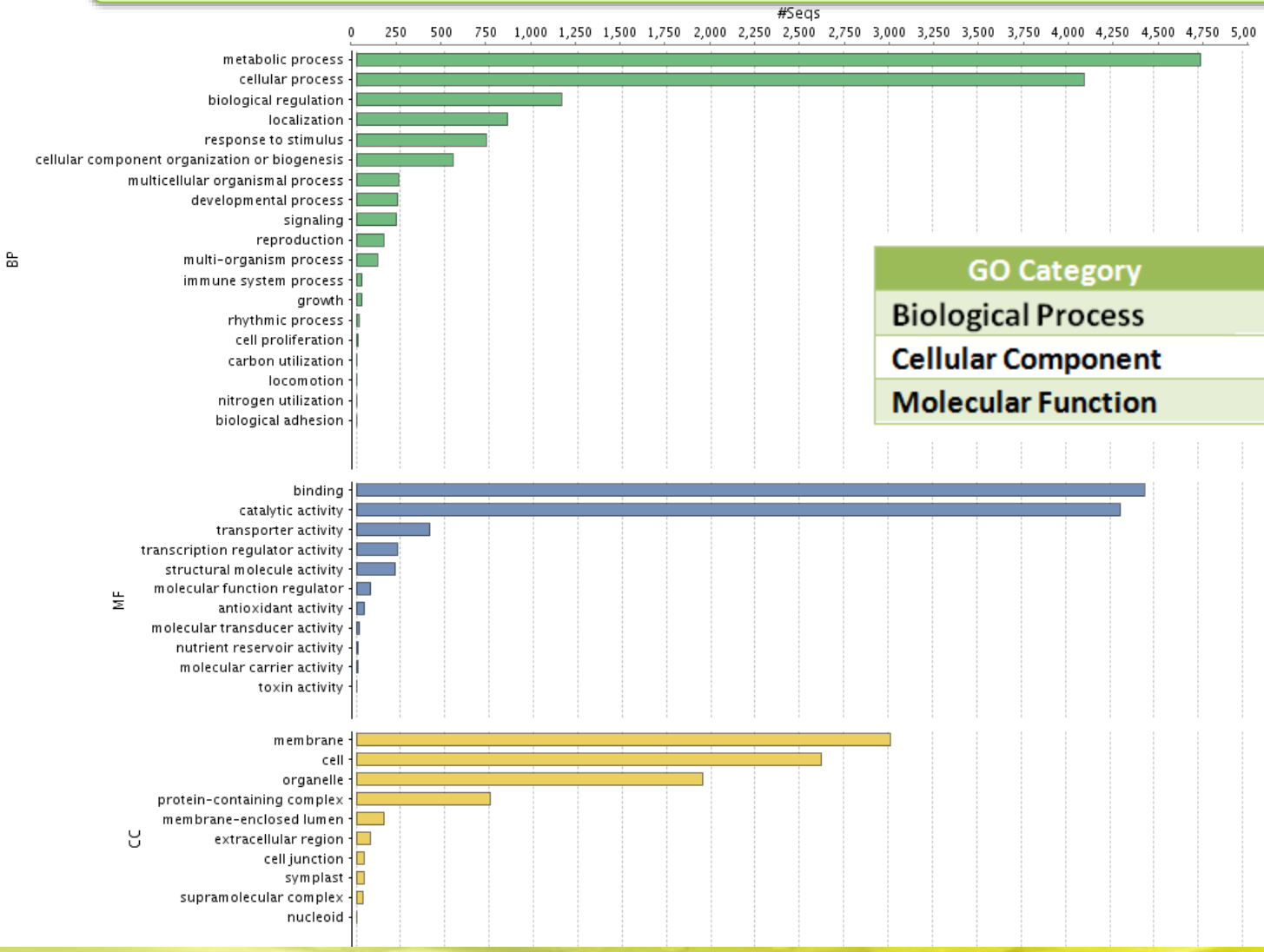
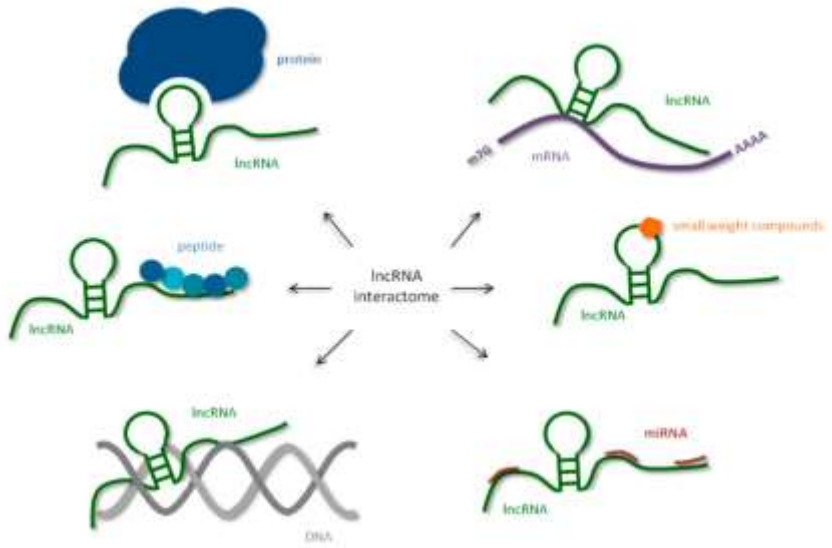
Functional analysis of cocoa transcripts-GO categories



GO Category	No. of Genes
Biological Process	6140
Cellular Component	4972
Molecular Function	7179

Long non-coding RNAs (LncRNAs) of cocoa in response to drought



Conserved lncRNA families of more than 10 members were identified

94 drought-responsive lncRNAs (35 up- and 59 down-regulated, respectively)

32 pairs are *cis*-NATs

Non-coding RNAs

Conserved lncRNA families: tRNA, snoR71 and LSU_rRNA accounted for >10 members

Small nucleolar RNA families (SNORD14, SNORD18, SNORD25, snoR71, snoR116) constituted the major categories of lncRNAs

miRNA 159, miR-395 , miR172, miR396, miR397, mir169, miR535, miR162, miR403,

Conclusions

Biomarkers (transcriptome-based RNA signatures, small non-coding RNAs, genic SSRs) to screen genotypes for water deficit stress tolerance

A panel of cocoa accessions are being screened for water stress tolerance utilizing the combination of biochemical, physiological and molecular features

Speed breeding of drought tolerant cocoa genotypes?

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हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद

Agrisearch with a human touch