

Introduction: Cassava is an important food crop of the *Euphorbiaceae* plant family. This diverse plant family includes other economically important crops and some undesirable species (**Table 1**).

Species	Value
Cassava (<i>Manihot esculenta</i>)	Food security crop
Castor bean (<i>Ricinus communis</i>)	Important oil crop
Rubber tree (<i>Hevea brasiliensis</i>)	Source for rubber production
Poinsettia (<i>Poinsettia pulcherrima</i>)	Important horticultural crop
Leafy Spurge (<i>Euphorbia esula</i>)	Undesirable invasive weed

Like most crop plants, growth and productivity is impacted by abiotic stress factors such as drought and cold. Some varieties of cassava are drought-tolerant but few, if any, are cold-tolerant. However, other species within the *Euphorbiaceae* family, such as leafy spurge, are extremely cold/freeze-tolerant. Leafy spurge also differs from cassava in that it carries a trait for vegetative reproduction from underground adventitious crown and root buds (**figure 1**). The divergence in evolution between these two related family members offers some interesting avenues for gaining a better understanding of conservation and diversity of genes leading to these inherent traits.

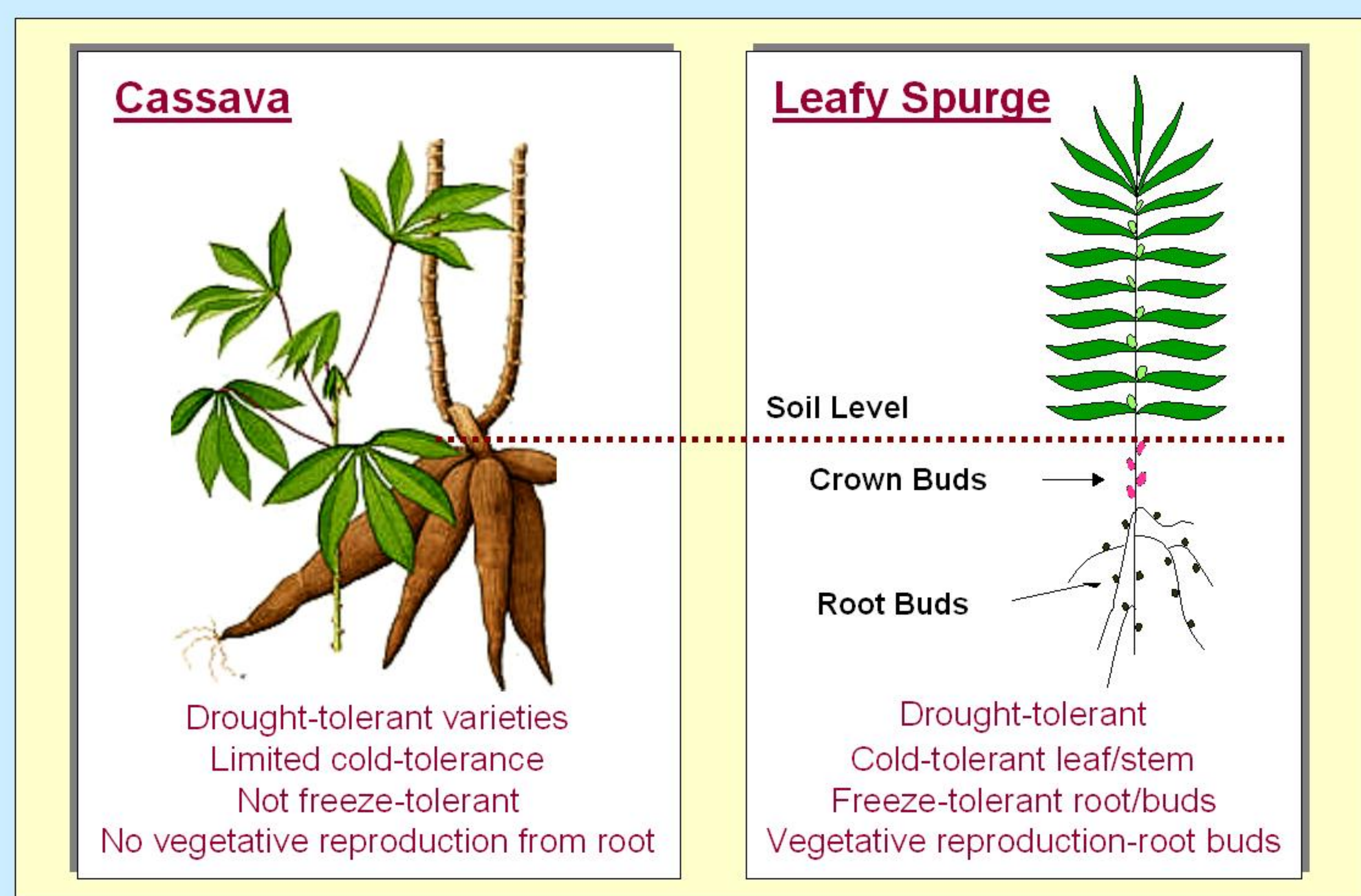


Figure 1. Comparison of cassava and leafy spurge plant anatomy and inherent genetic traits.

Monitoring gene expression and function is requisite to identifying signal perception and transduction pathways that drive the expression of genes harboring traits for drought- and cold/freeze-tolerance and vegetative reproduction. Currently, the most powerful approach for determining global gene expression is through microarray technologies. DNA microarray technology depends on the availability of Expressed Sequence Tags (ESTs; generated by single pass sequencing of expressed genes randomly picked from cDNA libraries). Scientists at CIAT, IITA, and USDA have realized the importance of using a genomics approach to compare expression profiles related to specific traits and are generating EST resources that can be utilized for the development of *Euphorbiaceae*-specific DNA microarrays (1). A list of the cDNA libraries currently being exploited or developed is shown in **Table 2**.

Species	Variety	Trait	Tissue-condition	Source
Cassava-	TME 117	Drought-tolerant	Whole Plant-control	IITA/USDA
	TME 117	Drought-tolerant	Whole plant-dehydrated	IITA/USDA
	TMS(4)2 1425	Drought-tolerant	Seedling-subtracted	IITA
	Mbra 685	CBB-tolerant	Stem-non inoculated	CIAT/UP
	Mbra 685	CBB-tolerant	Stem-not subtracted	CIAT/UP
	Mbra 685	CBB-tolerant	Leaf-subtracted	CIAT/UP
	SG107-35	CBB-resistant	Stem-not subtracted	CIAT/UP
	SG107-35	CBB-resistant	Stem-subtracted	CIAT/UP
	M Col 1522	CBB-susceptible	Stem-subtracted	CIAT/UP
	M Col 1522	CBB-susceptible	Stem-inoculated	CIAT/UP
	TME 3	CMD-resistant	Leaf/Stem-field exposure	CIAT/IBRC
	CM523-7	Starch content	Roots-high dry matter	CIAT/UP
	Mper 183	Starch content	Roots-low dry matter	CIAT/UP
Leafy Spurge-	LS001	Drought/Cold-tolerant	Root bud-3 day induced	USDA
	LS001	Drought/Cold-tolerant	Root bud-subtracted	USDA
	LS001	Drought/Cold-tolerant	Whole plant-stressed	USDA

This report describes the current status of progress towards the development of an EST-database for *Euphorbiaceae* and preliminary results showing how this resource can be used as a tool for monitoring gene expression within this diverse plant family.

Methods: Details pertaining to library construction, EST-database development, DNA array and RNA blot analysis were accomplished as described by Anderson *et al.* (1).

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Abbreviations: CIAT, *Centro Internacional de Agricultura Tropical*; IITA, *International Institute for Tropical Agriculture*; IBRC, *Iwate Biotech Research Center*; UP, *University of Perpignan*; CBB, cassava bacterial blight; CMD, cassava mosaic disease; EtBr, ethidium bromide.

Results & Discussion: There are ~18,000 quality ESTs in the *Euphorbiaceae* EST-database (~16,000 cassava and ~2,000 leafy spurge). Statistics of the ESTs analyzed within the current EST-database, that are being used to develop DNA arrays, are listed in **Table 3**.

Library Source	# of Contigs	# of ESTs in Contigs	Singleton ESTs	# of Unigenes
CBB/Starch	1875	9218	3825	5700
CMD	500	2995	1005	1505
Leafy Spurge induced bud	246	642	1172	1418

To attain the full potential of DNA microarray technologies, we will need to increase the number of available unigenes. Producing additional ESTs from existing and newly developed cDNA libraries of *Euphorbiaceae* is addressing this need. Two new normalized cDNA libraries for cassava TME 117 and one for leafy spurge contain $> 6.2 \times 10^5$ and 1.6×10^6 clones, respectively. Preliminary statistics for ESTs obtained from each of the cassava TME 117 libraries are listed in **Table 4**.

Category	Control	Dehydration-stressed
# of preliminary sequences	192	192
# of good quality sequence	157 (~82%)	174 (~91%)
Redundancy	8.9%	5.2%
# of contigs	10	8
# of singletons	133	157
# of unique sequences	143	165
# of unique sequences common to both libraries	8	8
# of unique sequences identified as unknowns	27 (~19%)	38 (~23%)

Since many orthologous genes from cassava and leafy spurge cross-hybridize, the developing *Euphorbiaceae* EST-database has proven useful in monitoring gene expression using heterologous systems (1). **Figure 2** shows how EST resources isolated from different family members can be used to monitor expression profiles of transcripts involved in numerous metabolic pathways. This approach could provide a powerful mechanism for unlocking the genetic diversity within *Euphorbiaceae* and should provide new knowledge important for understanding signaling and transduction pathways involved in drought- and cold-tolerance or vegetative reproduction.

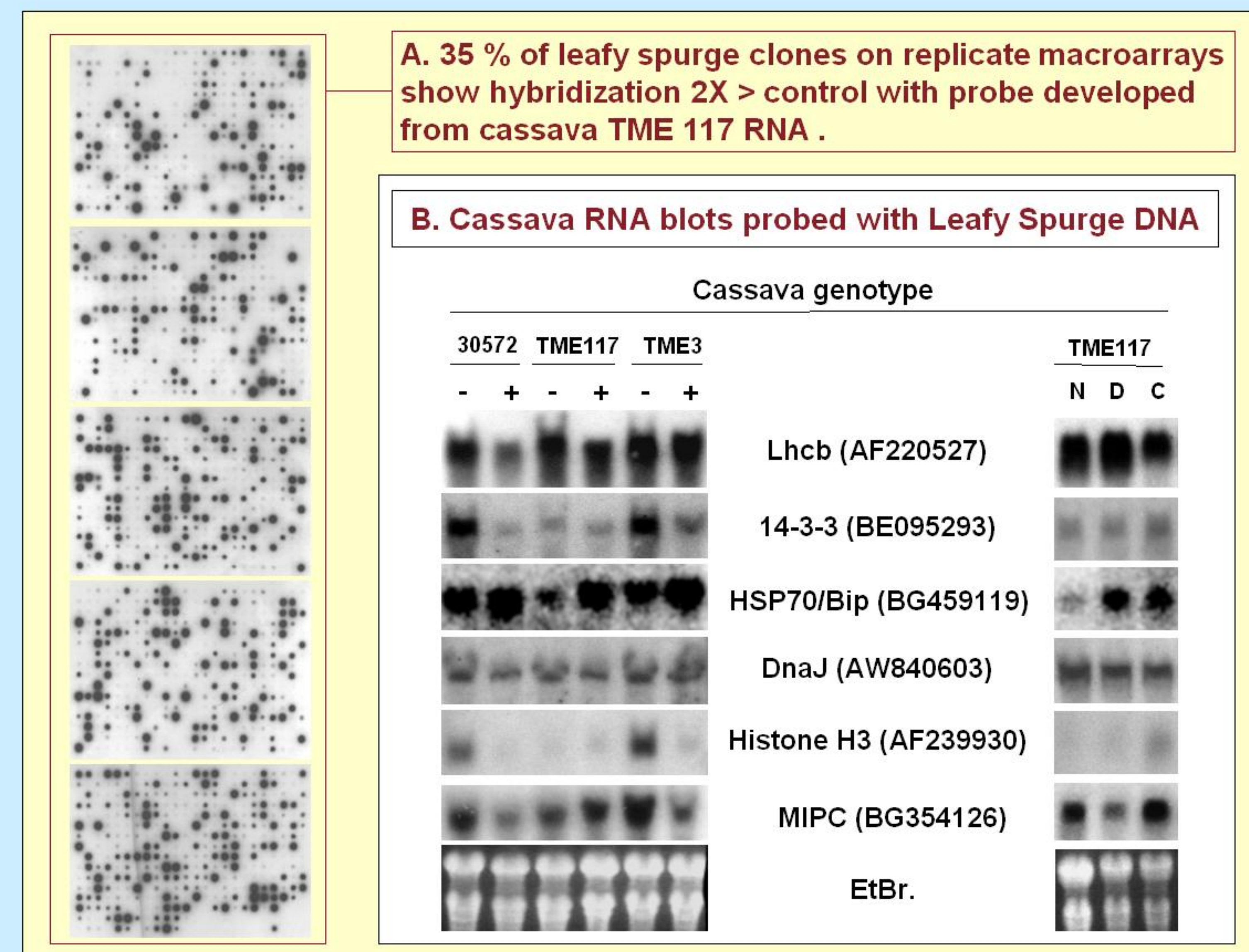


Figure 2: DNA macroarray (A) containing leafy spurge DNA was probed with target cDNA developed using total RNA from cassava dehydration-stressed young leaf. RNA blots (B) representing cassava young leaf tissue were probed with labeled cDNAs from leafy spurge. Treatments are indicated as: - and + represent 4 hours at 25° or 42°C, respectively; N, normal leaf; D, 7-day dehydration; C, 30 hours cold (4-6°C). Total RNA/lane = 20 µg. GenBank accession # for leafy spurge clones used to develop probe is indicated in parenthesis.

Conclusions:

1. Progress towards an *Euphorbiaceae* EST-database and microarrays is well on its way and is already providing promising results.
2. Sequence information and expression profiles from these projects are important for unlocking the genetic diversity within the *Euphorbiaceae* plant family and should help identify pathways and markers important to desired genetic traits such as drought- and cold-tolerance.
3. Further development of the *Euphorbiaceae* EST-database is needed to exploit the full potential offered through DNA microarray technologies.

References:

Anderson, J.V., Delseny, M., Fregene, M.A., Jorge, V., Mba, C., Lopez, C., Restrepo, S., Soto, M., Piegu, B., Verdier, V., Cooke, R., Tohme, J., Horvath, D.P. (2004) An EST Resource for Cassava and Other Species of *Euphorbiaceae*. *Plant Molecular Biology*, Invited Paper for Special Issue (accepted 12-1-03).

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