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Insight on Pathogen Defense Mechanisms in the Sugarcane Transcriptome

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ABSTRACT

Recognition of pathogen and activation of defense mechanisms is a common feature known from all multicellular organisms. Among higher plants, systemic acquired resistance (SAR) is known to activate pathogenesis-related (*PR*) genes after recognition of the pathogen mediated by a resistance (*R*) gene. Both gene classes (*R* and *PR*) represent the main mechanism against biotic and sometimes also abiotic stresses. Therefore, the identification of SAR metabolism components is an important concern regarding plant breeding. Sugarcane (*Saccharum officinarum*) is a major tropical and subtropical crop, grown in more than 120 countries, being especially important due to its value for the production of bioethanol, constituting a renewable energy source, besides the sugar production. The present work brings an overview on sugarcane resistance and pathogenesis-related genes, regarding their structure, abundance and role in the plant-pathogen metabolic pathway and also regarding their distribution, as compared with rice. For this purpose a collection of 282,818 expressed sequences tags have been evaluated to identify *R* and *PR* genes as well as important factors identified in the classic plant-pathogen metabolic KEGG pathway using rice full length cDNA as seed-sequences. The identified sugarcane genes have been also used to screen four SuperSAGE libraries with 8,787,313 tags, allowing the identification of the main activated and repressed genes under abiotic stress (drought/salinity) conditions. The 1,460 identified genes have been plotted on a rice virtual karyotype inferring about their distribution, considering a putative synteny as a measure to infer about their relative position within rice and sugarcane chromosomes. The results revealed interesting insights on the variability and complexity of defense genes in sugarcane.

Keywords: bioinformatics, crosstalk among biotic and abiotic stresses, *PR*-genes, *R*-genes
Abbreviations: **ABA**, abscisic acid; **AS**, salicylic acid; **avr**, avirulence; **BLAST**, basic local alignment search tool; **EST**, expressed sequence tag; **HR**, hypersensitive response; **LRR**, leucine rich repeats; **NBS**, nucleotide binding site; **PR**, pathogenesis related; **R**, resistance; **ROS**, reactive oxygen species; **SAR**, systemic acquired resistance; **Ser/Thre-Kinase**, serine/threonine kinase; **ST**, signal transduction; **SuperSAGE**, super serial analysis of gene expression

INTRODUCTION

The prevalence of a disease constitutes an abnormal condition, affecting plant growth and impairing important physiological processes. As highlighted by FAO (2005), fungi, bacteria, virus and nematodes are the main disease agents, resulting in serious losses to agriculture and also native plants, reducing the productivity, nutritional value and overall quality of the produced biomass.

During the plant-pathogen co-evolution, plants develop a complex network of synergic mechanisms to defend against pathogen attack (Pinzón *et al.* 2009). Considering this complex response, one of the most important steps includes detection of the possible invaders by the plant, a step where Resistance (*R*) genes play a crucial role (Mofet 2009). This sensing involves the recognition of a pathogen gene product called avirulence (avr) factor by a matching *R* gene. The plant will be resistant and the pathogen growth and establishment will be impaired when both *avr* and *R* genes are compatible, leading to the so called hypersensitive response (HR) including local cell death to impair spreading of the pathogen (Jones and Dangl 2006). Besides this localized response, the HR activates a signal cascade which is able to establish resistance against a spectrum of different pathogens (Wang *et al.* 2005), corroborating the observations made at the beginning of the last century that plants, as animals, may be immunized against the attack a of given pathogen after infection by another pathogen (Chester 1933).

In the past decade many aspects of the systemic acquired resistance (SAR) have been elucidated (Ingle *et al.* 2006). The SAR pathway is also common in many incompatible plant-pathogen interactions (Park *et al.* 2010). As soon as the pathogen is detected, the plant induces a set of complex signal molecules that may activate defense proteins (Humphry 2010) or that may have direct antimicrobial effect, as it is the case of the pathogenesis-related (*PR*) genes (Durrant and Dong 2004) or alternatively the production of secondary metabolites that impair pathogen movement or growth within the plant tissues (Sparla *et al.* 2004; Benko-Iseppon *et al.* 2010).

Categories of *R* genes

Altogether *R* genes have been recently classified into five different groups or classes, defined by their conserved domains (CD; Bent 1996; Hammond-Kosak and Jones 1997; Ellis and Jones 2000).

The first class is represented by the *HM1* gene of maize that codes for a reductase able to inactivate toxins produced by the fungus *Helminthosporium carbonum* (Joahal and

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Briggs 1992), being the only *R* gene class where conserved domains are absent. A second class is represented by the *Pto* gene from tomato, that confers resistance against the bacterium *Pseudomonas syringae* pv. *tomato* – a pathogen that express the corresponding *avrPto* avirulence gene (Martin *et al.* 1993). The *Pto* gene is characterized by a Serine/Threonine-kinase (Ser/Thre-Kinase) domain, able to interact with the *avrPto* gene (Tang *et al.* 1999). This gene was also identified in other plants *viz. Arabidopsis thaliana*, *Phaseolus vulgaris* (Melotto *et al.* 2004) and *Saccharum officinarum* (Wanderley-Nogueira *et al*. 2007).

The third class is represented by genes bearing two domains: leucine-rich repeats (LRR) and nucleotide binding site (NBS) (Liu *et al.* 2004). This is the case of the *Rpm1* and *Rps2* genes from *A. thaliana* (Mindrinos *et al.* 1994), the *N* gene from *Nicotiana tabacum* (Whitham *et al.* 1994), *L6* from *Linum usitatissimum* (Lawrence *et al.* 1995), *Prf* from *Lycopersicon esculentum* (Salmeron *et al.* 1996) and *Rpg1* from *Hordeum vulgare* also found in *P. vulgaris*, *G. max* and *Vicia faba* (Brueggeman *et al.* 2008) The fourth *R* gene class codes for a membrane anchored protein composed by a LRR extracellular domain, a transmembrane region and a short intracellular tail in the C terminal. The *Cf* gene from *L. esculentum* is an example of this class, conferring resistance against *Cladosporium fulvum* (Jones *et al.* 1994; Dixon *et al.* 1996).

The *Xa21* gene from rice that confers resistance to the bacterium *Xanthomonas oryzae* pv. *oryzae* is a representative of the fifth class (Song *et al.* 1995; Wang *et al.* 1995). This gene encodes for an extracellular LRR domain (similar to the *Cf* gene), as well as a Ser/Thre-Kinase domain (similar to the *Pto* gene), suggesting an evolutionary ligation among different classes/domains in the genesis of plant *R* genes (Song *et al.* 1997).

Pathogenesis-related (*PR*) gene categories

The PR proteins comprise of pathogen-induced proteins being routinely classified into 17 families based on their biochemical and molecular biological properties, from PR-1 to PR-17 (van Loon *et al*. 2006).

Similarities among sequences and serologic or immunologic properties are the base of their classification (Van Loon *et al.* 1999). Functionally some PR proteins bear antifungal properties, as the chitinases (PR-3) and β-glucanases (PR-2) (Zhu *et al.* 1994), while others like PR-1 from *N. tabacum* (Atici and Nalbantoğlu 2003), *A. thaliana* (Metzler *et al.* 1991), *L. esculentum* (Tornero *et al.* 1997) and *Malus domestica* (Bonasera *et al.* 2006) present unknown phytochemical functions. Despite of that, this gene class is considered to be a typical SAR marker (Bonasera *et al.* 2006).

Most *PR* genes are expressed to a basal level under normal growth conditions, but are rapidly induced by pathogenic infections. It is notable that several *PR* genes are also regulated during development, for example during leaf senescence and pollen maturation, and also by environmental factors, as osmotic stress, cold and light (Broekaert *et al*. 2000; Zeier *et al*. 2004).

Some PR proteins, including PR-1, chitinases, and thaumatin-like proteins are expressed under cold stress in overwintering monocots, exhibiting antifreeze activities (Hon *et al*. 1995; Atici and Nalbantoğlu 2003; Griffith and Yaish 2004).

Many *PR* genes are constitutively expressed in given plant tissues (Velazhahan and Muthukrishnan 2003; Liu *et al*. 2004), indicating that at least some members of the PR proteins play important roles in plant development other than defense responses, as occur with PR-2 protein in *N. tabacum*, that play an important role in seed germination (Seo *et al.* 2008) . Consistent with this notion, it has been shown that a tobacco PR-2 protein plays a role in seed germination (Leubner-Metzger and Meins 2000; Leubner-Metzger 2005).

Although many *PR* genes have been identified in different plant species, recent research have focused on their molecular aspects by using them as indicators for pathogenic infections, salicylic acid (SA) signaling, and SAR (van Loon *et al*. 2006). Transgenic approaches were also employed to obtain information on the role of PR proteins in disease resistance response. As a result, it is unclear how the *PR* genes are regulated by environmental conditions in consonance with plant growth hormones, and to what extent the PR proteins affect plant development.

Hormones

Besides *R* and *PR* genes, hormones are also important signaling molecules, playing an important regulatory role in plant development and inducing the expression of many PR proteins. Such hormones are produced at specific sites and in low amounts, also being active in defense pathways, as it is the case of SA, jasmonic acid (JA) and ethylene (ET), considered to be the main molecules activating defense genes (Lu 2009; Divi *et al.* 2010).

Induction of genes encoding *PR-1, PR-2* (β-1,3-glucanase), and *PR-5* (thaumatin-like protein) requires SA signaling. In contrast, genes encoding *PR-3* and *PR-4* (both with endochitinase activities) are independent of SA signaling and depend on JA pathway. For example, in arabidopsis, SA and JA activate distinct sets of *PR* genes in an antagonistic pattern (Thomma *et al*. 1998).

The SA pathway is primarily linked to resistance to biotrophic pathogens i.e. organisms that feed and reproduce on living tissues. This is in contrast to JA and ET, which mediate resistance mostly against necrotrophic pathogens (organisms which kill their hosts and derive nutrients, live and multiply on dead tissue). This differentiation of defense signaling pathways suggest that plants detect differences between pathogen lifestyle and mode of infection. Genetic evidence for JA antagonism of SA signaling pathways is well documented, but emerging data suggest a more complex signaling network evoking both positive and negative regulatory interactions (Spoel *et al*. 2007; Lópes *et al*. 2008; Vlot *et al*. 2008).

SA is a strong inducer of *PR* genes, and particularly *PR-1* is traditionally used as a marker for SA-mediated defence (Gaffney *et al*. 1993). A different set of genes is activated by JA, such as *VSP2* and *PDF1.2* (Benedetti *et al*. 1995; Penninckx *et al*. 1998). The latter, *PDF1.2*, also responds to ET (Thomma *et al*. 2001). Lately, it has become apparent that plant growth hormones and modulation of developmental processes was not recognized earlier as being part of plant defense. A model proposed by Robert-Seilaniantz *et al*. (2007) showed that auxin and cytokinins promote biotrophic susceptibility by inducing necrotrophic resistance pathways via JA/ET. Plants have evolved mechanisms to suppress auxin signaling as a component of basal defense in order to hinder the invading pathogens from using the hormone as virulence factor (Navarro *et al*. 2006). In parallel, gibberellic acid (GA) induces necrotrophic susceptibility, by inducing the biotrophic SA resistance pathway (Robert-Seilaniantz *et al*. 2007).

However, most of these interactions hitherto remain to be proved. For example, the role of abscisic acid (ABA) in a plant stress context is complex. ABA is a well known component in abiotic stress responses, but has been shown recently to be important in defense to various pathogens, highlighting the known crosstalk among different stress types (Asselbergh *et al*. 2008; Schenke *et al.* 2011). For example, in the interaction among arabidopsis and *Leptosphaeria maculans*, ABA is important for resistance, wherein both callose dependent and independent pathways are present (Kaliff *et al*. 2007). Indeed, some transcription factors and signaling molecules are common molecular players in both biotic and abiotic stresses, common to multiple networks or involved in crosstalk between stress signaling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene as well as ROS (reactive oxygen species) signaling (Velázquez *et al.* 2011).

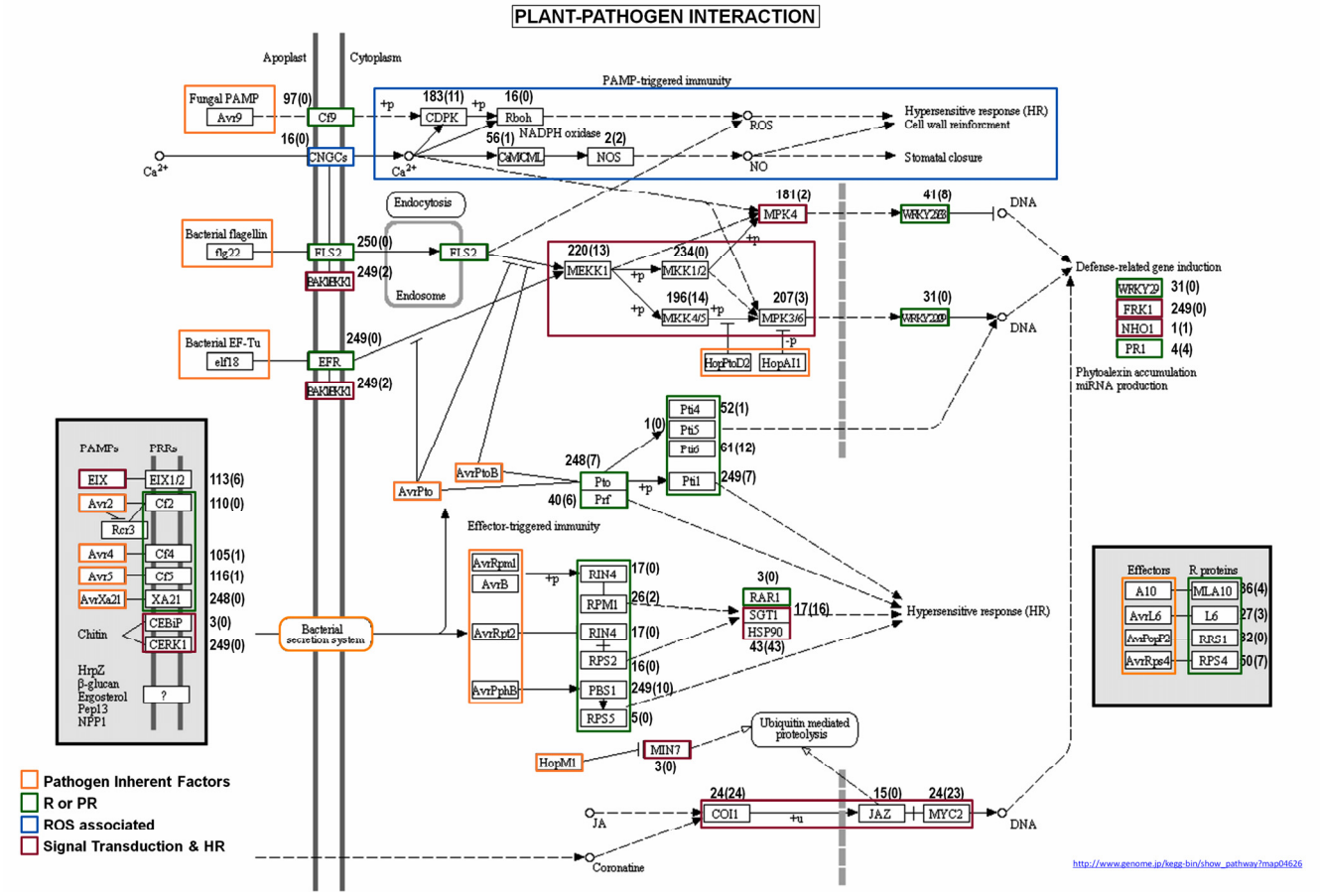

Fig. 1 Plant-pathogen interaction pathway available at KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg-bin/show_pathway?map04626) showing the number of elements found in sugarcane transcriptome for each gene type, followed by the number of exclusively aligned sequences (between parenthesis). Abbreviations: HR, hypersensitive response; PAMP: pattern associated to the pathogen; PR, pathogenesis-related; PaPRR: pattern recognition receptor; R, resistance; ROS, reactive oxygen species.

Reactive oxygen species (ROS)

ROS are produced by all aerobic organisms as a by-product of aerobic metabolism. A substantial increase in the intracellular concentration of ROS is generally associated with deleterious effects, including cell death by apoptosis or necrosis, in pathological conditions such as inflammation. The generation of ROS also appears to be required for many normal cellular functions, including transduction of cell surface receptor signaling (Woo and Rhee 2010).

Depending on the nature of the ROS, some are highly toxic and rapidly detoxified by various cellular enzymatic and non-enzymatic mechanisms. Whereas plants are fortified with mechanisms to combat increased ROS levels during abiotic stress conditions, in other circumstances plants appear to purposefully generate ROS as signaling molecules to control various processes including pathogen defense, programmed cell death, and stomatal behavior (Apel and Hirt 2004).

Altogether, *avr*, *R* and *PR* genes, as well as hormones and ROS are active during plant-pathogen interaction as illustrated in **Fig. 1**.

The sugarcane expressed sequence tag (EST) database

Many projects have been carried out aiming to identify expressed sequence tags (ESTs) in sugarcane, including initiatives in Brazil, South Africa, Australia, and France (Grivet and Arruda 2001; Perrin and Wigge 2002). Together these projects produced more than 300,000 sugarcane ESTs. In Brazil, the ONSA (Organization for Nucleotide Sequencing and Analysis; http://watson.fapesp.br/onsa/Genoma3.htm) consortium initiated the SUCEST (Sugarcane Expressed Sequence Tag Project; http://sucest.lad.dcc.unicamp.br/en/) – an initiative that generated about 238,000 ESTs distributed over 26 libraries from different tissues and across developmental stages. The data generated is still not completely evaluated and many gene families remain to be analyzed, as it is the case of the *PR* genes which are here evaluated for the first time. Despite of the considerable genetic variability among sugarcane germplasm revealed by molecular markers (e.g. Parida *et al.* 2010; Costa *et al.* 2011), few evaluations of the genetic diversity and complexity of this crop are available, especially regarding genes associated to the pathogen response.

The present evaluation brings an overview of the main sequences regarding plant-pathogen interaction in sugarcane, considering the up to date knowledge regarding the model plant rice. It also brings an insight on the expression of such sequences regarding the differential expression in sugarcane by Super-SAGE (Super Serial Analysis of Gene Expression) during abiotic stresses (drought and salinity), showing the dynamic relationship in the response and its modulation during different stress situations, explained by the crosstalk mechanism, i.e., the co-activation of genes among both biotic and abiotic stress types in an interaction.

MATERIALS AND METHODS

Rice sequences used as reference database to identify sugarcane candidates

In the present chapter, the first approach for the identification of sugarcane clusters associated with plant pathogen interaction was based on the metabolic pathway map046262 at KEGG (Kyoto Encyclopedia of Genes and Genomes; http://www.genome.jp/kegg/). For this purpose, KEGG sequences were downloaded and used in the search of the respective full length cDNA from rice that were subsequently used as reference-sequences. The selected

Table 1 Sugarcane clusters matching results to each gene of KEGG pathway. Showing number of matches to each seed sequence, number of clusters that aligned exclusively with corresponding gene, e-value, score, size in nucleotide (nt) and aminoacids (aa) and presence of conserved domains.

KEGG pathway		Sugarcane							
Class	**Gene**	**Matches up to e-5**	**Exclusive clusters**	**Best match**	**e-value**	**Score**	**Size (nt)**	**Size (aa)**	**Domain complete = CP incomplete = IC**
PR	*PR1*	3	4	lcl|CL2300-Ct1	3 e^{88}	322	1178	240	-
R	*Cf-2*	110	0	lcl|CL587-Ct3	1 e^{75}	177	2208	714	LRR (CP)
	Cf-5	116	1	lcl|CL10869-Ct1	9 e^{105}	205	994	311	LRR (CP)
	Cf-4	105	1	lcl|CL587-Ct3	4 e^{56}	122	2208	714	LRR (CP)
	Cf-9	96	0	lcl|CL587-Ct3	8 e^{36}	84.9	2208	714	LRR (CP)
	EFR	249	0	lcl|CL587-Ct3	4 e^{85}	150	2208	714	LRR (CP)
	FLS2	250	0	lcl|CL587-Ct3	1 e^{102}	207	2208	714	LRR (CP)
	Xa21	248	0	lcl|CL587-Ct3	1 e^{76}	89.0	2208	714	LRR (CP)
	Pto	248	7	lcl|CL3705-Ct1	0.0	623	1656	449	Ser-Thr Kin. (CP)
	PRF	40	6	lcl|CL11633-Ct1	3 e^{112}	322	948	294	-
	WRKY25	41	8	lcl|CL3534-Ct1	7 e^{68}	165	1424	279	WRKY (CP)
	WRKY29	31	0	lcl|CL4442-Ct1	4 e^{33}	140	784	108	-
	Pti1	249	7	lcl|CL1219-Ct3	0.0	419	1667	207	Prot.Kin. (c-IC)
	Pti 4	52	1	lcl|CL13275-Ct1	2 e^{47}	104	853	256	AP2 (n-IC)
	Pti5	1	0	lcl|CL13275-Ct1	5 e^{06}	49	853	256	AP2 (n-IC)
	Pti6	61	12	lcl|CL13048-Ct1	1 e^{30}	117	453	150	AP2 (n-IC)
	RIN4	17	0	lcl|CL11031-Ct1	5 e^{29}	81.3	905	233	-
	RPM1	26	2	lcl|CL4449-Ct1	1 e^{105}	85.4	3165	470	NBS (n-IC)/LRR (CP)
	RPS2	16	0	lcl|CL9468-Ct1	3 e^{19}	44	675	126	NBS (n-IC)
	RPS4	50	7	lcl|CL12379-Ct1	5 e^{73}	243	746	66	-
	RPS5	5	0	lcl|CL2439-Ct2	9 e^{07}	53.8	1261	374	NBS/LRR (CP)
	PBS1	249	10	lcl|CL4898-Ct1	0.0	602	1277	356	Prot.Kin. (CP)
	RAR1	3	0	lcl|CL20348-Ct1	4 e^{16}	63.4	685	223	CHORD (CP)
	MLA10	36	4	lcl|CL12379-Ct1	2 e^{80}	166	746	66	-
	L6	27	3	lcl|CL17937-Ct1	5 e^{45}	128	623	164	NBS (n-IC)
	RRS1	32	0	lcl|CL9540-Ct1	4 e^{85}	299	685	217	NBS (n-IC)
ST +HR	*CERK1*	249	0	lcl|CL2995-Ct1	8 e^{180}	398	1017	316	Prot.Kin. (CP)
	JAZ	15	0	lcl|CL1899-Ct2	8 e^{80}	266	1401	270	Tify super Fam. (CP)
	MPK4	181	2	lcl|CL681-Ct2	1 e^{174}	515	1554	375	Prot.Kin.-like (CP)
	FRK1	249	0	lcl|CL3705-Ct1	0.0	623	1656	449	Prot.Kin. (CP)
	EIX	113	6	lcl|CL587-Ct3	6 e^{46}	82.6	2208	714	LRR (CP)
	MYC2	24	23	lcl|CL3629-Ct1	4 e^{73}	189	1023	150	-
	MIN7	8	8	lcl|CL836-Ct4	0.0	397	1523	496	-
	HSP90	43	43	lcl|CL143-Ct3	0.0	593	2125	610	HTPase_c (CP)
	MPK3/6	207	3	lcl|CL4057-Ct1	0.0	797	1467	278	Prot. Kin. (CP)
	MKK1/2	234	0	lcl|CL3935-Ct1	1 e^{136}	319	1021	312	PKc_MAPKK (CP)
	COI1	24	24	lcl|CL464-Ct1	0.0	907	1789	390	-
	NHO1	1	1	lcl|CL15012-Ct1	4 e^{110}	354	1038	302	FGGY_N super Fam. (IC)
	BAK1/SER4	249	2	lcl|CL1733-Ct2	0.0	763	1916	276	Prot. Kin. (CP)
	MEKK1	220	13	lcl|CL7215-Ct1	3 e^{158}	534	1179	288	Prot. Kin. (CP)
	CEBiP	3	0	lcl|CL18653-Ct1	2 e^{69}	179	735	225	LysM (CP)
	MKK4/5	196	14	lcl|CL11106-Ct1	3 e^{47}	187	573	149	-
ROS	*STG1*	17	16	lcl|CL470-Ct2	0.0	270	1530	362	TPR (CP)
	CNGCs	16	0	lcl|CL2014-Ct2	0.0	648	1933	628	CAP_ED
	CDPKs	183	11	lcl|CL319-Ct6	0.0	1070	2194	535	Prot.Kin./EF-hand/EF-hand
	Rboh	16	0	lcl|CL1560-Ct2	0.0	653	1781	287	NOX_Duox
	CaMCML	56	1	lcl|CL59-Ct10	8 e^{106}	360	756	149	EF-hand/EF-hand
	NOS	2	2	lcl|CL12950-Ct1	2 e^{93}	245	775	195	GTPase_YqeH(n-IC)

Abbreviations: LRR = Leucine-rich repeat; CP = complete; Fam = family; IC = incomplete; Kin = Kinase; Prot = protein.

genes included 26 *R* or *PR*-genes, five ROS genes and 18 genes that act with signal transduction and are involved in the hypersensitive response, including hormones.

An additional analysis was performed using 17 *R*-genes previously compiled by Barbosa-da-Silva *et al*. (2005) and Wanderley-Nogueira *et al*. (2007) and 15 PR-genes searched by keyword, function and conserved domains at NCBI (PR-1: NP_179068; PR-2: NP_191285.1; PR-3: NP566426.2; PR-4: NP_187123; PR-5: NP_177641.1; PR-6: AAA16881; PR-7: NP_001234257; PR-8: CAM82810; PR-9: AAA34108; PR-10: ACF5101; PR-11: CAA01263; PR-12: P30230; PR-13: AEE35295; PR-14: CAA91436; PR-15: AAB561565.1; PR-16: NP_001061164).

Thus, a total of 81 full length cDNA sequences from *Oryza sativa* from the MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/) composed the reference-sequence database.

Analysis of the sequences obtained

For the identification of these gene analogs in sugarcane transcriptome, tBLASTx (Basic Local Alignment Tool) alignments were carried out against a local database [Clusterized ESTs from NCBI (National Center for Biotechnology Information) and SUCEST] including 282,878 sequences, using a cut-off value of e^{-5}.

Obtained sugarcane clusters were annotated and analyzed regarding score, e-values, sequence size and presence of conserved domains as shown in **Table 1**. For this purpose sugarcane clusters were translated using the TRANSLATE tool of Expasy (http://us.expasy.org/) and screened for conserved motifs with aid of the RPS-BLAST CD-search tool (Altschul *et al*. 1990). The best match for each gene was submitted to a BLASTx alignment in NCBI GenBank in order to confirm their putative function (results are available in http://150.161.22.10/sugarcane/all.fasta.html). A second manual analysis was also carried out, followed by an elimination of clusters that matched to more than one gene due to common domains. For this purpose, matching clusters to each

query sequences were annotated on a local database called 'non-redundant'.

Evaluation of genes associated to pathogen invasion under abiotic stress by Super-SAGE

SuperSAGE data was generated by our group as described by Molina *et al*. (2008) in collaboration with GenXPro GmbH (http://www.genxpro.info/) and Brazilian partners (CTC – The Center of Sugarcane Technology, http://www.ctcanavieira.com.br/; CETENE - Centro de Tecnologias Estratégicas do Nordeste, http://www.cetene.gov.br/home/index.php). The RNA samples were obtained from roots of selected sugarcane accessions (*Saccharum* spp.) under salinity (100 mM NaCl) or drought (after 24 h irrigation suppression) stresses in two distinct experiments:

(A) Salinity stress assay: acclimated plants of the clone RB931011 (referred as tolerant to salinity stress by the Brazilian RIDESA program of sugarcane breeding) were grown in a greenhouse (CETENE, Recife, Brazil) in pots containing washed sand and watered daily with Hoagland solution. For salinity stress application 100 mM NaCl was added to the mentioned solution. After salinity stress induction (30, 60 and 90 min) roots were collected from both, stressed and non-stressed (negative control) plants, that were immediately frozen in liquid nitrogen until processing and total RNA extraction. Equimolar amounts of each sample/time were assembled to form the bulks referred to the two SuperSAGE libraries (stressed and control).

(B) Drought stress assay: selected genotypes provided by the Center for Sugarcane Technology breeding program (CTC, Piracicaba, Brazil) were previously identified in a drought tolerance essay. Among them, four genotypes composed the drought sensitive group (CTC9, CTC13, SP90-3414 and SP90-1638) and another four regarded the drought tolerant group (CTC15, CTC6, SP83-2847 and SP83-5073). The plants were grown in 10-L containers up to three months of development when part remained irrigated and part were submitted to water suppression (24 h). Roots samples were collected, identified and frozen in liquid nitrogen until processing and total RNA extraction aiming to compose the four SuperSAGE libraries: TD: tolerant under stress; TC: tolerant control; SD: sensitive under stress and SC: sensitive control.

Unique tags (26 bp) differentially expressed (p-value 0.05) were identified using DiscoverySpace 4.01 software. All tags were submitted to BLASTn (score = 52; 100% of identity) against the previously identified *PR* and *R* sugarcane contigs dataset previously annotated using Uniprot-SwissProt database (BLASTx; e-value e^{-10}). All data were normalized (one million tags by library) and the fold change (FC) of a differentially expressed tag was calculated with the tag frequency in a stressed library in relation to the respective control (without stress). The normalized data matrix regarding the gene expression was analyzed using a hierarchical clustering approach with aid of the program Cluster 3.0. Dendrograms (using the weighted pair-group analysis) were generated using the TreeView program.

Anchoring sugarcane genes in the rice physical map

Considering the availability of a genome browser for the rice genome, all sugarcane ESTs and SuperSAGE tags were aligned against *O. sativa* non-redundant clusters anchored on virtual chromosomes from the MSU Rice Genome Annotation Project. For this purpose, the MegaBLAST tool was used with at least 80% identity or direct association with the differentially expressed SuperSAGE tags.

Although the BLAST algorithm was adjusted to make searches as sensitive as possible and considering that this tool only generates local alignments, to ensure that the anchored clusters in the rice genome represented real genes and not just similarity with random segments, the obtained data underwent to a second screening, which considered possible splicing sites, deletions and/or insertions, and the total length of the clusters (considering that the data should represent at least 30% of cluster size).

For better data organization, clusters that represented high similarity with the same region of the rice genome were grouped. The groupings are named in increasing order as GRn, where "GR" means group and "n" the group number. For the virtual karyotype representation a CorelDRAW12 graphic application was used; the rice chromosome information to the schematic representation was obtained from GRAMENE site (http://gramene.org/Oryza_sativa/Location/Genome). For the design of chromosomes, considering the high data amount to be anchored in the genome and the necessity of high resolution of bands, a random length of 200 mm was adopted for the larger chromosome (number 1); thus, for the band marking, each 1 mm corresponded to 126,344 bp. Moreover, clusters and groupings that schematically appeared very close to each other were indicated as a single band.

RESULTS AND DISCUSSION

The tBLASTx alignment in sugarcane database using the 49 KEGG pathway reference sequences returned 1,150 clusters, indicating that all members of this pathway are represented in sugarcane (**Fig. 1**). Among these clusters only 244 were non-redundant, aligning each with one corresponding reference sequence. Other 906 clusters presented repetitions, aligning with two or more reference sequences, confirming the excellent coverage that the existing Sugarcane EST databank comprises, including the most important representatives from different gene families. Regarding the exclusive 244 sugarcane clusters, 74 aligned with *PR* or *R*-genes, 14 aligned with ROS genes and 156 with signal transduction (ST) genes or genes involved in the HR (**Table 1; Fig. 1**). From these, 64% aligned with genes of the hypersensitive response, 30% with *R* and *PR* genes while 6% aligned with ROS. As reviewed by Benko-Iseppon *et al.* (2010) genes of the HR are associated with different stimuli, being activated not only due to pathogen perception, but also under abiotic stress, whilst many of them are constitutively active in low levels, also in the absence of any kind of stress, justifying their prevalence in the present evaluation. Thus, a growing number of evidences supports the notion that plant signaling pathways consist of elaborate networks with frequent crosstalk, thereby allowing plants to regulate both abiotic stress tolerance and disease resistance (Velázquez *et al.* 2011).

Most of the 169 clusters that aligned with two different genes were sequences bearing similar conserved domains

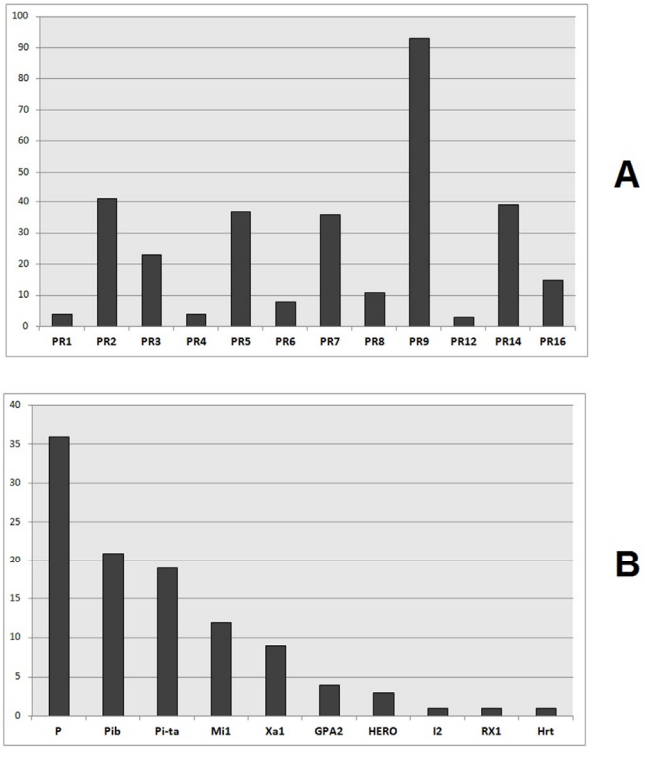

Fig. 2 Distribution of the 314 sugarcane clusters that aligned with *PR*-genes families (**A**) and of the 107 sugarcane clusters that aligned with *R*-genes with no repetitions (**B**).

like LRR, Kinases or NBS. This occurred among the *Pti4* and *Pti6*, sharing 50 clusters; *WRKY25* and *WRKY29* with 25 identical sequences, *Cf2* and *Cf5* with 7 and *ERF* and *Xa21* that shared three identical sugarcane clusters. Other 737 clusters repeat themselves from three to 20 times among the 49 genes presented in KEGG plant response pathway. This is an expected result also observed in a previous approaches (Barbosa-da-Silva *et al*. 2005; Wanderley-Nogueira *et al.* 2007), due to similar CDs existing in *R* genes among angiosperms.

The 16 classes of *PR* genes search returned 314 non-redundant sugarcane clusters. Among them, only four sequences matched with two different reference sequences (*PR-4* and *PR-6*) and the remaining 310 pertained exclusively to one *PR*-gene (**Fig. 2A**).

The most abundant category was *PR-9* (with 93 representatives), including factors classified as peroxidases (**Fig. 2A**). They contribute to plant disease resistance in several ways including (i) the strengthening of plant cell walls through the deposition of lignin, which is thought to be a general defense mechanism against a broad spectrum of pathogens; and (ii) the production of toxic radicals such as hydrogen peroxide (Van Loon and Van Strien 1999).

PR-2 was the second most frequent gene category with 41 members (**Fig. 2A**), regarding a group of β-1,3-glucanases, whose role in disease resistance is often related to their glucanase activity. PR-2 proteins can either directly impair the growth of a fungus by hydrolyzing β-1,3/1,6-glucans within fungal cell walls or by releasing short glucan fragments from pathogen cell walls, which can also be recognized by plants and further induce plant defense responses (Ebel and Cosio 1994; Van Loon and Van Strien 1999).

PR-5 (thaumatin-like), 7 (endoproteinase) and 14 (lipid transfer protein) were also well represented with 37, 35 and 39 matches, respectively (**Fig. 2A**). The PR-5 family belongs to the thaumatin-like proteins with homology to permatins that permeabilize fungal membranes (Vigers *et al.* 1991). Some members of this family have been shown to possess antifungal activity, particularly against oomycetes. Basic PR-5 proteins (osmotin) are induced in tobacco and tomato in response to osmotic stress (Singh *et al.* 1987). PR-7 acts as an endoproteinase. Because lysis of fungal cell walls often requires degradation of cell wall proteins in addition to hydrolysis of chitin and glucan, it seems reasonable to assume that PR-7 serves as an accessory to antifungal action (Goldman and Goldman 1998), while lipid-transfer proteins (LTPs) (PR-14) exhibit both antifungal and antibacterial activity, exerting their effect at the level of the plasma membrane of the target micro-organism. Their relative diversity in sugarcane reinforces previous observations (Garcia-Olmedo *et al.* 1995).

Regarding the additional 17 *R*-genes analysis, other 118 non-redundant sugarcane clusters presented matches with *O. sativa* reference sequences. Eleven of these clusters aligned with two different *R*-genes (*Mi1* and *GPA2*; *HERO* and *Pi-ta*) and 107 aligned exclusively with 10 genes (**Fig. 2B**).

The genes *P* of flax, *I2* and *Mi* of tomato, *HERO*, *Rx1* and *GPA2* of potato *Hrt* of arabidopsis (Cooley *et al.* 2000) and *Pib*, *Pi-ta* and *Xa1* of rice are all members of the NBS-LRR family, that was the most represented class in the sugarcane transcriptome (**Fig. 2B**); this class common feature is the presence of (i) leucine-rich repeats which play a direct role in protein-protein specific recognition event; LRR motif contains 23-25 aa with a consensus sequence (LxxLxxLxLxxNxLt/sgxIpxxLG); (ii) a nucleotide-binding site that is highly conserved among plants, and is similar to mammalian CED-4 and APAF-1 proteins which are involved in apoptosis (Chinnaiyan *et al.* 1997) and usually signal for programmed cell death playing a role in activetion of downstream effectors (Bryan *et al.* 2000), and (iii) coiled-coil (CC) or a TIR (Toll Interleukine-Like domain), involved in signal transduction during many cell processes.

Curiously, these 10 genes that confer resistance against bacteria, fungi, virus or nematodes, despite of sharing common domains, presented exclusive alignments with sugarcane sequences while the other 54 *R*-genes (considering kinases, NBS-LRR, LRR and other families) presented alignments with common clusters.

A high number of NBS-LRR class representatives was observed (490 clusters) as compared with other classes previously observed by Barbosa-da-Silva *et al*. (2005) and Wanderley-Nogueira *et al.* (2007) and were expected because most of *R*-genes pertain to this family that play a crucial role in plant defense.

Other 443 sequences aligned with the seven remaining *R*-genes previously identified among the 1,150 sugarcane clusters matching with KEGG pathway. As occurred in most genes selected from the KEGG database, repetitions probably appeared because of the occurrence of different combinations of the same domains (16 genes present LRR, 15 genes present NBS and 20 genes are protein kinases, for example).

Most of the sugarcane clusters that appeared as best matches presented the same conserved domains complete. In the few cases where domains were not identified, their function was confirmed using BLASTx at NCBI.

Considering the taxonomic affiliation of the best matches, as expected, their alignment occurred with monocot species, among them, 74% aligned with *Sorghum bicolor*, 15% with *Zea mays*, 9% with *O. sativa*, and 1% with *Saccharum* hybrid, with a single alignment to a non-angiosperm (1% to *Laccaria bicolor*, a mushroom). Considering the high abundance of rice sequences in data banks, it would be expected that most matches would belong to sequences of this species. On the other hand, the higher similarity to sorghum and maize is justified by their taxonomic proximity to sugarcane within *Panicoidae* of the *Poaceae*, where sorghum and sugarcane grouped together in the Andropogoneae (Watson and Dallwitz 1992; Bouchenak-Khelladi *et al.* 2008).

Pathogen defense genes and abiotic stress Super-SAGE assay

Despite of the focus of the described SuperSAGE being the transcriptome of plants under abiotic stress [drought: water suppression; salinity (100 mM NaCl)], many R (**Table 2**) and PR (**Table 3**) transcripts have been identified, an expected result since biotic and abiotic signaling pathways share multiple nodes and their outputs have significant functional overlap, and are able to be cross-activated. Examples of such events were presented by Chini *et al.* (2004) while establishing enhanced expression of the CC-NBS-LRR gene, *ADR1* (a broad spectrum disease resistance gene, with serine/threonine protein kinases domain) that conferred tolerance to drought.

Tags associated to plant *R* transcripts representing the major NBS-LRR class were observed to be differentially expressed (**Fig. 3A**). Usually, R proteins can directly interact with a product of the avirulence gene of the pathogen or indirectly by guarding another protein that is the target of the avirulence protein. Moreover, they can detect pathogen associated molecular pattern (PAMPs) and participate in activation of the innate immune system that will protect the host from infection, or even in degradation by a pathogen toxin. Another important class, the recognition receptors pattern (RRP), which include receptor protein kinases implicated in PAMP perception were also presented in the SuperSAGE results. Thus some tags annotated as receptor-like kinases (RLK), mainly LRR, were also differentially expressed (**Fig. 3A**). Receptor kinases play important roles in cell signaling being responsible for the information about the cell surroundings. This activation may be justified by the fact that some abiotic stresses open a door to pathogen invasion.

Considering **Fig. 3A**, the cluster I showed overexpressed tags in salinity-tolerant and in drought-sensitive genotypes. Examples of annotated R genes with high FC are represented by the cluster I were COI1 (an important jasmonate receptor) and TIR1 (auxin receptor) are included.

Table 2 Sugarcane upregulated SuperSAGE tag ($P < 0.05$) under abiotic stress-related to sugarcane contigs annotated to *R* gene products.

Tag	Contig	R protein	Description	Stress*	Chromosome
Sg232385	lcl|CL4049Ctg-1	FBL3	F-box/LRR-repeat prot. 3	Salt	-
Sg309748	lcl|CL3705Ctg-2	HERK1	Recep. prot. kin. HERK 1	Salt	6
Sg309667	lcl|CL444Ctg-9	RKL1	Recep. kin.	Salt	-
Sg313217	lcl|CL1Ctg-731	RLK	Recep. kin.	Salt	12
Sg27138	lcl|CL4449Ctg-1	RPM1	Disease resistance prot. RPM1	Salt	11
Sg350189	lcl|CL1621Ctg-1	SRF3	Prot. strubbelig recep. fam. 3	Salt	2
Sg58453	lcl|CL1621Ctg-1	SRF3	Prot. strubbelig recep. fam. 3	Salt	2
Sg145047	lcl|CL138Ctg-2	TRXH	Thioredoxin H-type	Salt	-
Sg14055	lcl|CL3962Ctg-2	APK1B	Prot. kin. APK1A, chloroplastic	Drought	9
Sg41941	lcl|CL3193Ctg-1	LRR	LRR-repeat (LRR) prot.	Drought	3
Sg284448	lcl|CL6862Ctg-1	LRR-RLK	LRR-repeat recep. kin.	Drought	-
Sg171649	lcl|CL357Ctg-3	MYB4	Myb-related prot. Myb4	Drought	-
Sg307209	lcl|CL1086Ctg-2	RLK	Recep. kin.	Drought	5
Sg108593	lcl|CL2628Ctg-2	RLK	Recep. kin.	Drought	2
Sg164846	lcl|CL3899Ctg-1	SERK1	Somatic embryogenesis recep.-kin.1	Drought	-
Sg32183	lcl|CL1415Ctg-1	SRF3	Prot. strubbelig recep. fam. 3	Drought	-
Sg168565	lcl|CL6296Ctg-1	STPK	Ser/Thr prot. kin.	Drought	3
Sg86044	lcl|CL8140Ctg-1	STPK	Ser/Thr prot. kin.	Drought	2
Sg108292	lcl|CL13024Ctg-1	TIR1	Transport inhibitor resp. 1 prot.	Drought	-
Sg159973	lcl|CL955Ctg-1	TRX2	Thioredoxin H-type 2	Drought	-
Sg51485	lcl|CL138Ctg-2	TRXH	Thioredoxin H-type	Drought	-
Sg85924	lcl|CL1Ctg-1314	BRL1	Ser/Thr prot. kin. BRI1 1	Salt/ Drought (S)	12
Sg67140	lcl|CL2957Ctg-1	CLV1	Recep. prot. kin. CLAVATA1	Salt/ Drought (S)	3
Sg112945	lcl|CL262Ctg-8	COI1	Coronatine-insensitive prot. 1	Salt/ Drought (S)	-
Sg359087	lcl|CL2155Ctg-1	DSK1	LRR-recep. Ser/Thr prot. kin.	Salt/ Drought (S)	1
Sg177002	lcl|CL7232Ctg-1	EXS	LRR-repeat / prot. kin. EXS	Salt/ Drought (S)	2
Sg55869	lcl|CL2503Ctg-4	FER	Recep. prot. kin. FERONIA	Salt/ Drought (S)	1
Sg261220	lcl|CL3311Ctg-1	HSL1	Recep. prot. kin. HSL1	Salt/ Drought (S)	8
Sg269246	lcl|CL592Ctg-6	HT1	Ser/Thr prot. kin. HT1	Salt/ Drought (S)	6
Sg29268	lcl|CL9800Ctg-1	LRR-RLK	LRR recep. prot. kin.	Salt/ Drought (S)	4
Sg136128	lcl|CL3699Ctg-2	MYB1	Myb-related prot. Hv1	Salt/ Drought (S)	-
Sg2721	lcl|CL1056Ctg-3	RLK	Recep. kin.	Salt/ Drought (S)	5
Sg165539	lcl|CL1Ctg-731	RLK	Recep. kin.	Salt/ Drought (S)	12
Sg242481	lcl|CL1963Ctg-3	TIR1	Transport inhibitor resp. 1 prot.	Salt/ Drought (S)	-
Sg327823	lcl|CL12575Ctg-1	EXS	LRR-repeat recep. kin. EXS	Salt/ Drought (T)	1
Sg202457	lcl|CL7304Ctg-1	MEE39	LRR recep. Sr/Th-prot. kin. MEE39	Salt/ Drought (T)	9
Sg321931	lcl|CL1750Ctg-1	STPK	Ser/Thr prot. kin.	Salt/ Drought (T)	10
Sg276480	lcl|CL3066Ctg-1	STPK	Ser/Thr prot. kin.	Salt/ Drought (T)	4
Sg285898	lcl|CL16830Ctg-1	WAK4	Wall-associated recep. kin. 4	Salt/ Drought (T)	-
Sg90440	lcl|CL1963Ctg-2	AFB2	Transport inhibitor resp. 1 prot.	Drought (T)	4
Sg112939	lcl|CL6590Ctg-2	AFB2	Transport inhibitor resp. 1 prot.	Drought (T)	-
Sg69207	lcl|CL900Ctg-4	APK1A	Prot. kin. APK1A, chloroplastic	Drought (T)	6
Sg161113	lcl|CL5649Ctg-1	APK2B	Prot. kin. 2B, chloroplastic	Drought (T)	10
Sg104430	lcl|CL1Ctg-1314	BRL1	Ser/Thr prot. kin. BRI1 1	Drought (T)	12
Sg185147	lcl|CL262Ctg-8	COI1	Coronatine-insensitive prot. 1	Drought (T)	-
Sg84672	lcl|CL9670Ctg-1	CRK19	Cysteine-rich recep. prot. kin. 19	Drought (T)	11
Sg106653	lcl|CL5650Ctg-1	CRK26	Cysteine-rich recep. prot. kin. 26	Drought (T)	-
Sg328269	lcl|CL14146Ctg-1	CTR1	Ser/Thr prot. kin. CTR1	Drought (T)	10
Sg249703	lcl|CL3947Ctg-1	CTR1	Ser/Thr prot. kin. CTR1	Drought (T)	-
Sg78151	lcl|CL3521Ctg-1	HT1	Ser/Thr prot. kin. HT1	Drought (T)	9
Sg32977	lcl|CL592Ctg-6	HT1	Ser/Thr prot. kin. HT1	Drought (T)	16
Sg139561	lcl|CL4853Ctg-1	LECRKA4.3	Lectin recep. kin. prot.	Drought (T)	10
Sg150666	lcl|CL11059Ctg-1	LRR RLK	LRR-repeat recep. kin.	Drought (T)	2
Sg197538	lcl|CL1856Ctg-2	LRR RLK	LRR-repeat recep. kin.	Drought (T)	8
Sg42130	lcl|CL2271Ctg-1	LRR RLK	LRR-repeat recep. kin.	Drought (T)	3
Sg258732	lcl|CL8133Ctg-1	LRR RLK	LRR-repeat recep. kin.	Drought (T)	-
Sg126264	lcl|CL2866Ctg-2	LRR	LRR-repeat (LRR) prot.	Drought (T)	-
Sg84166	lcl|CL2090Ctg-3	LRR-RLK	LRR-recep. prot. kin.	Drought (T)	11
Sg134899	lcl|CL2439Ctg-2	LRR UBL	LRR-ubiquitin Fam. prot.	Drought (T)	10
Sg81195	lcl|CL2439Ctg-2	LRR UBL	LRR-ubiquitin Fam. prot.	Drought (T)	10
Sg205102	lcl|CL3699Ctg-1	MYB1	Myb-related prot. Hv1	Drought (T)	-
Sg95975	lcl|CL357Ctg-3	MYB4	Myb-related prot. Myb4	Drought (T)	-
Sg164597	lcl|CL9007Ctg-1	NAK	Ser/Thr prot. kin. NAK	Drought (T)	6
Sg44998	lcl|CL5525Ctg-1	PUB34	U-box domain-containing prot. 34	Drought (T)	6
Sg157473	lcl|CL1296Ctg-5	RLK	Recep. like kin.	Drought (T)	5
Sg13069	lcl|CL4024Ctg-1	RLK	Recep. like kin.	Drought (T)	1
Sg16260	lcl|CL7850Ctg-1	RLK	Recep. like kin.	Drought (T)	12
Sg190665	lcl|CL289Ctg-2	SERK2	Somatic embryogen. recep. kin. 2	Drought (T)	1
Sg255086	lcl|CL1356Ctg-1	SRF3	Prot. strubbelig recep. Fam. 3	Drought (T)	7
Sg47088	lcl|CL586Ctg-1	SRF7	Prot. strubbelig recep. Fam. 7	Drought (T)	-
Sg273272	lcl|CL2666Ctg-1	SRF8	Prot. strubbelig recep. Fam. 8	Drought (T)	1
Sg103811	lcl|CL19234Ctg-1	STPK	Ser/Thr prot. kin.	Drought (T)	-

Table 2 (cont.)

Tag	Contig	R protein	Description	Stress*	Chromosome
Sg254990	lcl|CL3066Ctg-1	STPK	Ser/Thr prot. kin.	Drought (T)	4
Sg186844	lcl|CL1963Ctg-3	TIR1	Transport inhibitor resp. 1 prot.	Drought (T)	-
Sg64443	lcl|CL955Ctg-1	TRX2	Thioredoxin H-type 2	Drought (T)	-
Sg51480	lcl|CL138Ctg-7	TRXH	Thioredoxin H-type	Drought (T)	-
Sg169613	lcl|CL218Ctg-3	TRXH	Thioredoxin H-type	Drought (T)	-

* Stress [Salt = salinity: 100 mM NaCl; drought: 24 h after water suppression]. Key for abbreviations: Fam. = family; Kin. = Kinase; LRR = Leucine-rich repeat; Prot = protein; recep. = receptor; resp = response; S = susceptible; T = tolerant

Jasmonate signaling plays a critical role in protecting plants from pathogens or insect attack and in limiting damage from abiotic stresses (Hu *et al.* 2009). The phytohormone auxin has been implicated in developmental plant processes, including apical dominance, tropic responses, vascular development, organ patterning, flower and fruit development. Kepinski and Leyser (2005) provided evidence for a role of auxins in plant defense responses and suggested cross-talk between auxin, abiotic stress and biotic stress signaling pathways. Curiously, the same tags were underexpressed in the drought-tolerant library when compared with the respective control. The clusters II and IV presented up-regulated tags in drought-tolerant genotypes, but downregulated in the other comparisons. Examples of tags with this behavior are CRK19 and CRK26. Cysteine-rich Receptor-like Kinases (CRKs) have been suggested to play important roles in the regulation of pathogen defense and programmed cell death and CRK is part of plant Receptor-like kinases (RLK), a group of conserved signaling components that regulate developmental programs and responses to biotic and abiotic stresses (Wrzaczek *et al.* 2010). Some overexpressed RLK members were observed in cluster III (also in the others clusters). Tags of cluster III seem to be related to both stresses in a general manner. RLK represents nearly 2.5% of arabidopsis protein coding genes (Shiu and Bleecker 2001). Others RLK members observed were SERK1 (cluster V); SERK2 (cluster IV) and SRF3 (cluster I, II and V), SRF7 (cluster II, III) and SRF8 (cluster II).

In relation to the differentially expressed SuperSAGE tags associated with pathogenesis-related proteins presented in the root transcriptome of sugarcane after abiotic stress induction (**Fig. 3B**), some families were observed [PR-2 (Glucan endo-1,3-beta-glucosidase), PR-3 and PR-4 (chitinase), PR-5 (thaumatin-like), PR-6 (proteinase inhibitor), PR-9 (peroxidase), PR-14 (LTP)]. Potential chitinase tags were observed in many clusters (I, II, IV, V, VI). Liu *et al.* (2004) suggested that the class IV chitinase PmCh4A was involved in the defense response of western white pine to infection and also abiotic stress factors, besides their primary role in plant defense (these enzymes degrade the major component of fungal cell walls, β-1,4-linked polymers of *N*-acetyl-d-glucosamine). Additionally, some chitinases were also components of plant defense against higher concentrations of specific heavy metals, showing that their biological role is complex and more than expected (Békésiová *et al.* 2007). Another PR family observed in all clusters is the peroxidase group (PER1, PER2, PER3, PER4, PER12, PER15, PER30, PER35, PER36, PER51, PER52, PER72; **Fig. 3B**). Manandhar *et al.* (1999) presented findings showing that the accumulation level of peroxidase transcripts (and some PR-proteins: PR-1, PR-2, PR-3, PR-4 and PR-5) were higher in rice in response to *Bipolaris sorokiniana* and UV light than in response to avirulent isolate of *Pyricularia oryzae*.

Additional PR components observed in association with SuperSAGE tags (**Fig. 3B**) and abiotic stress were:

a) Cytosolic Ascorbate Peroxidase 1 (cAPX1; tag in cluster IV): Davletova *et al.* (2004) demonstrated that in the absence of the cytosolic H_2O_2-scavenging enzyme APX1, the entire chloroplast H_2O_2-scavenging system of *A. thaliana* collapses, H_2O_2 levels increase, and protein oxidation with programmed cell death occurs. On the other hand, cAPX2 (cluster IV and VI) products showed to be involved in flooding stress responses in young soybean seedlings

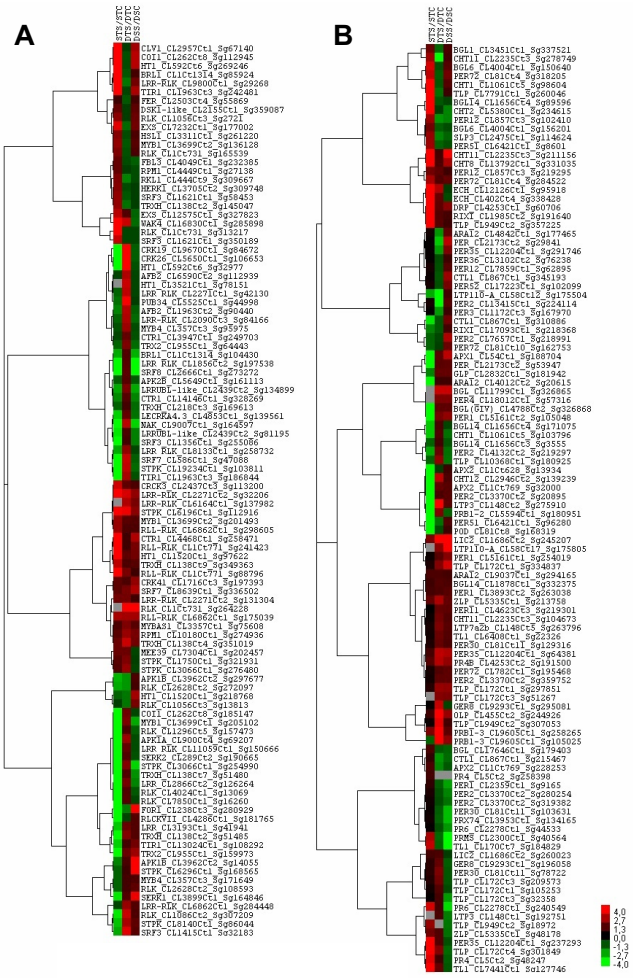

Fig. 3 Hierarchical clustering (Cluster3.0) of up-regulated (red) and down-regulated (green) SuperSAGE tags (P < 0,05) related to R (**A**) and PR classes (**B**) using FC (fold change) of gene expression ratios (experimental/control) under the tested conditions [STS/STC: Salinity-tolerant (100 mM NaCl)/control; DTS/DTC: drought-tolerant/control; DSS/DSC: drought-sensible/control]. The tag number and the contig ID is given together with the gene product.

(Shi *et al.* 2008);

b) Thaumatin – PR-5 (tags in clusters I, II, IV, V, VI, VII): transgenic plants with constitutively higher expression of the *GbTLP1* [cotton (*Gossypium barbadense* L.) thaumatin-like protein gene] showed enhanced resistance against different stress agents, particularly, *Verticillium dahliae* and *Fusarium oxysporum*, increasing also tolerance against some abiotic stresses including salinity and drought (Munis *et al.* 2010);

c) Germin-like proteins (tags in clusters IV, V, VII): GLP constitute a ubiquitous family of plant proteins that seem to be involved in many developmental and stress-related processes. Berna and Bernier (1999) showed that expression of the wheat germin gene was also stimulated by some abiotic stresses, especially the heavy metal ions Cd^{2+}, Cu^{2+} and Co^{2+};

d) Glucan endo-1,3-beta-glucosidase – PR-2 (synonym: [beta]-1,3-glucanase; tags in clusters I, IV, V, VI): this

Table 3 Sugarcane upregulated SuperSAGE tags ($P < 0.05$) under abiotic stress*, related to sugarcane contigs annotated to *PR* gene products.

Tag	Contig	PR protein	Description	Stress*	Chromosome
Sg89596	lcl|CL1656Ctg-4	BGL14	Glucan endo-1,3-beta-glucosidase 14	Salt	6
Sg156201	lcl|CL4004Ctg-1	BGL6	Glucan endo-1,3-beta-glucosidase 6	Salt	2
Sg234615	lcl|CL5380Ctg-1	CHT2	Chitinase 2	Salt	5
Sg9165	lcl|CL2359Ctg-1	PER1	Peroxidase 1	Salt	-
Sg102410	lcl|CL857Ctg-3	PER12	Peroxidase 12	Salt	4
Sg280254	lcl|CL3370Ctg-2	PER2	Peroxidase 2	Salt	5
Sg319382	lcl|CL3370Ctg-2	PER2	Peroxidase 2	Salt	5
Sg103631	lcl|CL81Ctg-11	PER30	Peroxidase 30	Salt	1
Sg8601	lcl|CL6421Ctg-1	PER51	Peroxidase 51	Salt	8
Sg44533	lcl|CL2278Ctg-1	PR6	Pathogenesis-related Prot. 6	Salt	1
Sg40564	lcl|CL2300Ctg-1	PRMS	Pathogenesis-related Prot. PRMS	Salt	1
Sg134165	lcl|CL3953Ctg-1	PRX74	Peroxidase 1	Salt	5
Sg114624	lcl|CL2475Ctg-1	SLP3	Serine-type peptidase 3	Salt	6
Sg184829	lcl|CL170Ctg-7	TL1	Thaumatin-like Prot. 1	Salt	10
Sg196058	lcl|CL9293Ctg-1	GER8	Germin-like Prot. 8	Salt/ Drought (T)	1,3
Sg260023	lcl|CL1686Ctg-2	LIC2	Lichenase-2 (Fragment)	Salt/ Drought (T)	5
Sg192751	lcl|CL148Ctg-1	LTP3	Non-specific lipid-transfer Prot. 3	Salt/ Drought (T)	11
Sg78722	lcl|CL81Ctg-11	PER30	Peroxidase 30	Salt/ Drought (T)	1
Sg237293	lcl|CL12204Ctg-1	PER35	Peroxidase 35	Salt/ Drought (T)	4
Sg48247	lcl|CL5Ctg-2	PR4	Pathogenesis-related Prot. 4	Salt/ Drought (T)	-
Sg240549	lcl|CL2278Ctg-1	PR6	Pathogenesis-related Prot. 6	Salt/ Drought (T)	1
Sg127746	lcl|CL7441Ctg-1	TL1	Thaumatin-like Prot. 1	Salt/ Drought (T)	10
Sg105253	lcl|CL172Ctg-1	TLP	Thaumatin-like Prot.	Salt/ Drought (T)	-
Sg209573	lcl|CL172Ctg-3	TLP	Thaumatin-like Prot.	Salt/ Drought (T)	12
Sg32358	lcl|CL172Ctg-3	TLP	Thaumatin-like Prot.	Salt/ Drought (T)	12
Sg301849	lcl|CL172Ctg-4	TLP	Thaumatin-like Prot.	Salt/ Drought (T)	12
Sg18972	lcl|CL949Ctg-2	TLP	Thaumatin-like Prot.	Salt/ Drought (T)	-
Sg48178	lcl|CL5335Ctg-1	ZLP	Zeamatin-like Prot.	Salt/ Drought (T)	3
Sg188704	lcl|CL54Ctg-1	APX1	L-ascorbate peroxidase 1 cytosolic	Drought	3
Sg13934	lcl|CL1Ctg-628	APX2	L-ascorbate peroxidase 2 cytosolic	Drought	7
Sg32000	lcl|CL1Ctg-769	APX2	L-ascorbate peroxidase 2 cytosolic	Drought	-
Sg20615	lcl|CL4012Ctg-2	ARA12	Subtilisin-like protease	Drought	4
Sg326868	lcl|CL4788Ctg-2	BGL(GIV)	Glucan endo-1,3-beta-glucosidase GIV	Drought	1
Sg326865	lcl|CL11799Ctg-1	BGL	Glucan endo-1,3-beta-glucosidase GVI	Drought	1
Sg3555	lcl|CL1656Ctg-3	BGL14	Glucan endo-1,3-beta-glucosidase 14	Drought	6
Sg171075	lcl|CL1656Ctg-4	BGL14	Glucan endo-1,3-beta-glucosidase 14	Drought	6
Sg103796	lcl|CL1061Ctg-5	CHT1	Chitinase 1	Drought	-
Sg139239	lcl|CL2946Ctg-2	CHT12	Chitinase 12	Drought	-
Sg181942	lcl|CL2832Ctg-1	GLP	Germin-like Prot.	Drought	1,3
Sg275910	lcl|CL148Ctg-2	LTP3	Non-specific lipid-transfer Prot. 3	Drought	-
Sg53947	lcl|CL2173Ctg-2	PER	Peroxidase	Drought	-
Sg105048	lcl|CL5161Ctg-2	PER1	Peroxidase 1	Drought	1
Sg20895	lcl|CL3370Ctg-2	PER2	Peroxidase 2	Drought	5
Sg219297	lcl|CL4132Ctg-2	PER2	Peroxidase 2	Drought	3
Sg57316	lcl|CL18012Ctg-1	PER4	Peroxidase 4	Drought	6
Sg96280	lcl|CL6421Ctg-1	PER51	Peroxidase 51	Drought	8
Sg168319	lcl|CL81Ctg-8	POD	Peroxidase 15	Drought	-
Sg180951	lcl|CL5594Ctg-1	PRB1-2	Pathogenesis-related Prot. PRB1-2	Drought	10
Sg180925	lcl|CL10368Ctg-1	TLP	Thaumatin-like Prot.	Drought	3, 9, 11
Sg177465	lcl|CL4842Ctg-1	ARA12	Subtilisin-like protease	Drought (S)	3
Sg310886	lcl|CL867Ctg-1	CTL1	Chitinase-like Prot. 1	Drought (S)	9
Sg345193	lcl|CL867Ctg-1	CTL1	Chitinase-like Prot. 1	Drought (S)	9
Sg175504	lcl|CL58Ctg-12	LTP110-A	Non-specific lipid-transfer Prot. 3	Drought (S)	-
Sg29841	lcl|CL2173Ctg-2	PER	Peroxidase	Drought (S)	-
Sg62895	lcl|CL7859Ctg-1	PER12	Peroxidase 12	Drought (S)	-
Sg224114	lcl|CL13415Ctg-1	PER2	Peroxidase 2	Drought (S)	-
Sg218991	lcl|CL7657Ctg-1	PER2	Peroxidase 2	Drought (S)	8
Sg167970	lcl|CL1172Ctg-3	PER3	Peroxidase 3	Drought (S)	6
Sg291746	lcl|CL12204Ctg-1	PER35	Peroxidase 35	Drought (S)	4
Sg76238	lcl|CL3102Ctg-2	PER36	Peroxidase 36	Drought (S)	-
Sg96280	lcl|CL6421Ctg-1	PER51	Peroxidase 51	Drought (S)	8
Sg102099	lcl|CL17223Ctg-1	PER52	Peroxidase 52	Drought (S)	2
Sg162753	lcl|CL81Ctg-10	PER72	Peroxidase 72	Drought (S)	1
Sg168319	lcl|CL81Ctg-8	POD	Peroxidase 15	Drought (S)	-
Sg218368	lcl|CL17093Ctg-1	RIXI	Xylanase inhibitor Prot. 1	Drought (S)	2, 11
Sg3555	lcl|CL1656Ctg-3	BGL14	Glucan endo-1,3-beta-glucosidase 14	Drought (T)	6
Sg171075	lcl|CL1656Ctg-4	BGL14	Glucan endo-1,3-beta-glucosidase 14	Drought (T)	6
Sg103796	lcl|CL1061Ctg-5	CHT1	Chitinase 1	Drought (T)	-
Sg95918	lcl|CL12126Ctg-1	ECH	Chitinase 12	Drought (T)	4
Sg338428	lcl|CL402Ctg-4	ECH	Chitinase 12	Drought (T)	4
Sg192751	lcl|CL148Ctg-1	LTP3	Non-specific lipid-transfer Prot. 3	Drought (T)	11
Sg219297	lcl|CL4132Ctg-2	PER2	Peroxidase 2	Drought (T)	3
Sg180925	lcl|CL10368Ctg-1	TLP	Thaumatin-like Prot.	Drought (T)	3, 9, 11

* Stress [Salt = salinity: 100 mM NaCl; drought: 24 h after water suppression].

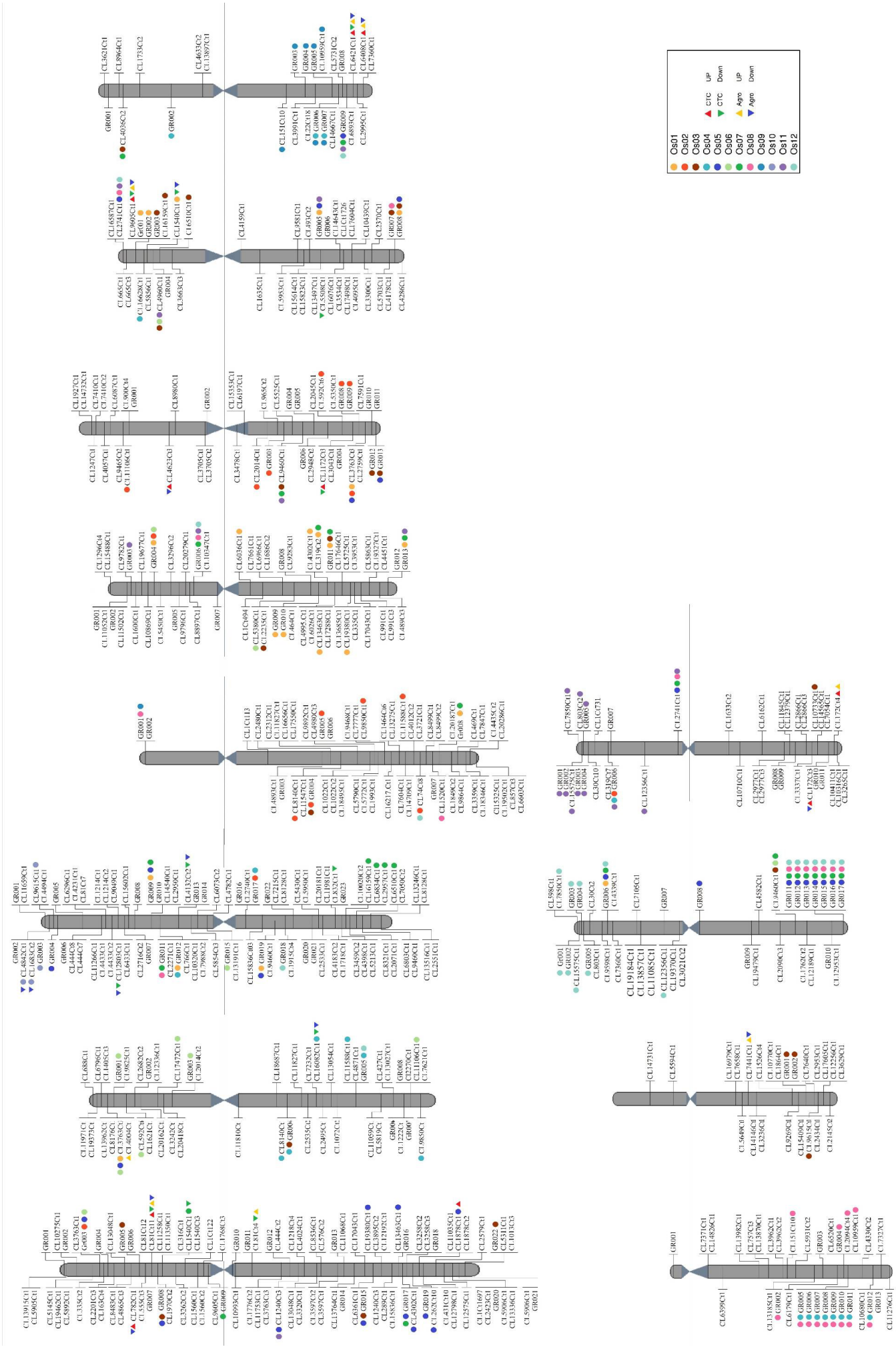
Os01
Os02
Os03
Os04
Os05
Os06
Os07
Os08
Os09
Os10
Os11
Os12
CTC UP
CTC Down
Agro UP
Agro Down

Fig. 4 (previous page) ***In silico*** **FISH.** Schematic representation of clusters/groupings that were anchored in the rice genome based on BLAST similarity results. Colored circles next to the clusters/grouping names correspond to the synteny events between chromosomes; each one of the 12 chromosomes was identified with a different color, as showed in the legend. Triangles and inverted triangles indicate the similarity of expressed tags (resulted from SuperSAGE) with clusters/grouping; the form colors and senses correspond to the library and regulation type, according the legend.

enzyme acts by reducing the influx of solutes into the membrane vesicles during freezing and thereby reduces osmotic stress and vesicle rupture during thawing (Hincha *et al.* 1997);

e) subtilisin-like protein: the salinity stress response in arabidopsis requires a subtilisin-like serine protease (AtS1P), related to membrane-localized b-ZIP transcription factor, AtbZIP17. Liu *et al.* (2007) observed that salinity stress induced a signaling cascade involving the processing of AtbZIP17, its translocation to the nucleus and the up-regulation of salinity stress genes;

f) Non-specific lipid-transfer protein (nLTP; tags in clusters III, IV, V, VII): LTP is another protein family involved in plant stress response (Jung *et al.* 2005; Sarowar *et al*. 2009), having the ability to transfer phospholipids between a donor and an acceptor membrane (when this activity is not specific, the peptides are called non-specific lipid transfer protein).

Besides the valuable identification of important genes associated with abiotic response, as osmoprotectants (Silva *et al.* 2011) by SuperSAGE, the here observed presence of PR genes during abiotic stress induction was also confirmed by Kido *et al.* (2010), that presented a functional review of antimicrobial peptides and an overview of SuperSAGE transcriptional profile of defensin (PR-12), thionin (PR-13) and LTP (PR-14) in libraries of some important crops (cowpea, soybean and sugarcane), again confirming their influence in mechanisms regarding biotic and abiotic genes. SuperSAGE allowed also the generation of a comprehensive panel of the differentially expressed kinases under biotic and abiotic stresses in cowpea (*Vigna unguiculata*), revealing their association with both kinds of stress (Kido *et al.* 2011). Such crosstalk interactions are evident in many differential expression profiling assays and indicate that in the future few genes may be useful to induce a myriad of responses, maybe useful for tolerance/resistance increase in crop plants.

Anchoring sugarcane sequences in the rice virtual karyotype

Beyond the BLAST algorithm parameters (score, e-value and percentage of identity) other features were considered (as the probable splicing sites, putative insertions, deletions and cluster full length in bp) for anchoring 73.22% of the 1,460 sugarcane clusters studied in the rice virtual karyotype (**Fig. 4**), in an attempt to infer about their relative position regarding possible synteny and colinearity among sugarcane and rice chromosomes.

Flowering plants originated approximately 200 million years ago (MYA; Wilkstrom *et al.* 2001) and subsequently diverged into several lineages. The Poaceae family arose about 60 MYA and diverged into different species mainly due to genome-wide amplification (Copley *et al.* 2001), in tandem gene duplication and events of local chromosome changes (Kondrashov *et al.* 2002). It is not surprising that 1,069 sugarcane clusters anchored in some region of the rice chromosomes, and one could assume that this occurred since both are members of Poaceae family; so the evolutionary proximity of rice and sugarcane means that consistent levels of homology may be expected among both species.

Vincentz *et al.* (2004) performed a comparison between sugarcane, rice and arabidopsis transcriptomes and found that 70.5% of sugarcane sequences were similar to arabidopsis (suggesting that their genes probably encoded essential angiosperm functions) and 81.6% had significant match with rice genome, so, these 11.1% sequences represents putative monocot specific material. Additionally, the other 18.4% of sugarcane sequences may correspond to gene losses in rice or fast-evolving sequences that diverged substantially. This can be the reason because 25.88% of our sugarcane clusters did not align to any region of the rice genome.

Sugarcane clusters appeared anchored in all segments of rice chromosomes. The rice chromosomes present heterochromatic regions (Cheng *et al*. 2001), but due to the limitations to sequence those regions, they are not linearly represented along the virtual karyotype, justifying the anchoring of many sugarcane sequences around the centromeres. Additionally, as expected, several sequences clustered along the genome, with some chromosomes rich in resistance genes (e.g. chromosomes 1 and 3) while other regions were relatively poor regarding the evaluated genes (e.g. chromosomes 4, 8, 10 and 12).

Clustering of *R* and *PR* genes confirms the existing theory that a common genetic mechanism has been involved in their evolution. Most resistance genes have been demonstrated to reside in clusters (Kanazin *et al*. 1996) as reported in maize (Dinesh-Kumar *et al*. 1995), lettuce (Maisonneuve *et al.* 1994), oat (Rayapati *et al.* 1994), flax (Ellis *et al.* 1995) and chickpea (Benko-Iseppon *et al.* 2003). The formation of gene clusters is in general associated with duplication processes followed by diversification through pressure from the pathogen or the environment, in the case of *R* genes and *PR* respectively. It is interesting to note that in short arms of chromosomes 4, 9 and 10 were anchored only one, one and two sequences respectively, which corroborates data from classical cytogenetics indicating that these areas reside in the vicinity of heterochromatic regions (Cheng *et al.* 2001). The distribution of clusters in regions highlighted by classical cytogenetics as heterochromatin, probably indicate the presence of euchromatic "islands" throughout the chromosome, closely related to high levels of expression (Yasuhara and Wakimoto 2006).

Some clusters presented association with centromeric regions or nearby existing repeats, as occurred in chromosomes 1, 2, 3, 5, 6, 7, 9 and 11. The presence of these clusters in regions of low gene expression activity is probably due to the alternation of euchromatin and heterochromatin around the centromere (Yan and Jiang 2007), another point to consider is the probability of modifications in histones H3 and H4, which allow gene transcription in this area. Also, previous works have shown that gene transcription may occur near the centromeres of rice chromosomes 8 and 3, so at least some elements could be transcribed in this area (Yan *et al*. 2006).

Regarding the number of sugarcane sequences similar to rice genome per chromosome, it was observed that chromosomes 1 and 3 presented the highest number of anchored sequences, matching 133 and 124 respectively, while the chromosome 10 presented the lowest number of aligned sugarcane sequences (**Fig. 5**).

Twelve clusters presented similarities with distinct segments in the same chromosome, being considered as duplications, as shown in **Table 4** and also indicated by color dots in **Fig. 5**. Those duplicated copies tend to diverge due to mutations and may specialize or optimize to play slightly different roles (Alberts *et al*. 1997). In rice one duplication event is assumed for each 20 million years in regard to its supposed ancestor, including the previous separation of cereals such as sorghum, maize and the Triticeae (Thiel *et al.* 2009). Regarding the duplicated segments considering the entire genome, 117 clusters could be identified in at least two distinct chromosomes. Different of what was cited before, repetitions in distinct chromosomes resulted from events of duplication, accidental translocations and sequence divergence, allowing functional diversification (Wendell 2000; Thiel *et al.* 2009). There are also evidences that trans-

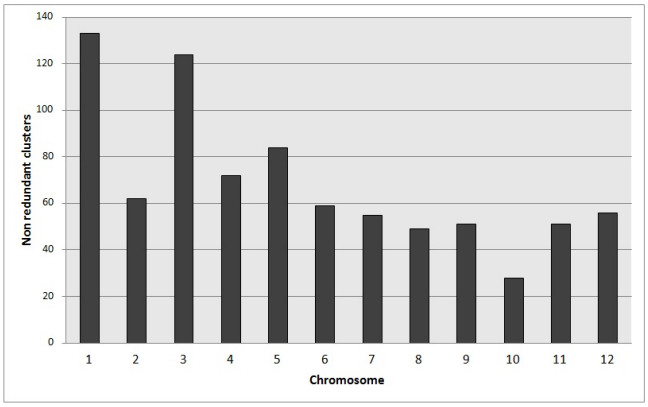

Fig. 5 Number of sugarcane clusters that aligned in each rice chromosome.

Table 4 Sugarcane clusters that presented similarities with distinct segments in a same rice chromosome.

Chromosome	Cluster	Position	
		Start	End
1	CL13048Contig1	6813508	6813684
		26733776	26733608
2	CL19373Contig1*	969864	970285
		981312	981732
3	CL13191Contig1*	17681689	17681192
		17702859	17702476
	CL2713 Contig1 e Contig 2	510781	512759
		997006	999145
	CL2740Contig1	22702577	22703551
		31482289	31481744
	CL8128Contig1	28750774	28748635
		35477873	35477018
7	CL15614Contig1*	21122766	21122970
		21130188	21130392
9	CL13870Contig1	10155655	10156233
		10366331	10366736
	CL22Contig10	18514955	18514293
		18525801	18525139
		18537260	18536598
		21015489	21015930
	CL22Contig8	18515301	18514527
		18526115	18525376
		18537574	18536835
		21015124	21015815
11	CL12189Contig1	26771155	26770944
		26790178	26789967
		26871113	26870902

positions outbreaks could be activated by severe environmental biotic or abiotic stress (Levin and Moran 2011).

Regarding synteny evidences, it was observed that chromosome 1 shares more similar regions with chromosome 5; chromosome 2 with chromosomes 4 and 6; chromosome 7 with 1 and 3; chromosome 9 with 8 and 4 and chromosome 10 presented synteny only with chromosome 3. On the telomeric region of chromosome 3 short arm a grouping of sequences could be observed, showing synteny with chromosome 10. The same occurred when the telomeric region of chromosome 11 short arm was analyzed, presenting a cluster of sequences showing synteny with the telomeric region of the chromosome 12 short arm.

Still regarding the analysis of duplication events, a large in tandem repetition was evident in the long arm of chromosome 9, represented by the groups GR005 to GR011. Previous reports suggest that once duplicated, in tandem repetitions may extend rapidly through events of unequal crossing over, what could confer some advantage (Alberts *et al*. 1997), in the present case, a higher diversity of genes associated to resistance and stress responses.

A remarkable degree of genome conservation has been established in comparative genetic mapping experiments for the Poaceae family, although genome sizes vary as much as 40-fold between some of the species, and despite the fact that they diverged as long as 60 million years ago (Gale and Devos 1998). Genetic mapping experiments in allohexaploid wheat revealed that most gene sequences are triplicated on the A, B and D genomes. Furthermore, the three sets of the seven homeologous chromosomes show overall colinearity. Evidence of a few translocation events was, however, also found (Devos and Gale 1993). Within the Triticeae tribe, extensive colinearity was established, for example, for the homeologous chromosomes of wheat, as compared with *Triticum monococcum*, *Triticum tauschii* and *H. vulgare*, and consensus maps were developed (Van Deynze *et al.* 1995; Dubcovsky *et al*. 1996). Microsynteny and colinearity were also observed in other angiosperms when compared to other distant related organisms, as it was the case of regions rich in factors associated with pathogen response chickpea and arabidopsis (Benko-Iseppon *et al.* 2003)

Considering the small size of the rice genome as compared with sugarcane, it is clear that higher levels of redundancy are expected in the sugarcane genome. Despite of that, the present evaluation may be valuable for the use of the identified genes for sugarcane breeding, since they may indicate putative linked gene markers for mapping purposes in sugarcane, especially considering most clustered regions here identified.

SuperSAGE tags BLAST against rice chromosomes

As result of drought and salinity superSAGE experiments, 76 tags matching resistance genes were upregulated when compared to non-stress conditions and 79 *PR* genes superSAGE tags candidates equally obtained from experiments submitted to abiotic stress were identified as upregulated. A considerable number of tags exposed to abiotic stress as drought and salinity aligned with *R* and *PR* genes including 115 sugarcane sequences that appeared as upregulated if compared to analysis in non-stressed conditions. It is known that both pathogen attack and abiotic stress may trigger a diverse array of plant defense-related genes involved in HR, which is characterized by necrotic lesions resulting from localized host cell death at the site of infection and also activating defense responses in uninfected parts of the plant, expressing so called SAR (Wang *et al*. 2010).

Considering the SuperSAGE output, 63% of *R*-genes candidates (28 tags) presented high similarities with rice genome segments. Matches in all chromosomes could be annotated, although chromosomes 1, 2 and 10 were those that presented higher number of anchored superSAGE tags. In the same way, 78.48% of *PR*-gene candidates (62 tags) matched alignments to rice genome regions, uncovering chromosomes 1, 3 and 6 as the most represented. Although most sequences anchored in rice chromosomes, 37% of the tags regarding *R*-genes and 21.6% of the *PR* ones did not present alignment with any region of the rice genome. They probably represent regions resulted from duplication events followed by divergent evolution (mutations), that are common in large genomes and may be a source of new genetic products that share common domains but may have distinct functions (Alberts *et al*. 1997). It is interesting also to consider that the SuperSAGE tags are expected to anchor at the 3′UTR region that are often outside the most conserved gene regions, maybe also bearing most frequent establishment of new mutations, when compared with conserved domains or folding regions of the gene.

In contrast, the chromosome 7 presented the lower number of alignments, matching only one *R*-gene and one *PR*-gene tag. Moreover, the search for similarities in the rice genome showed that four *PR* superSAGE tags could be identified anchoring in more than one chromosome. Both tags Sg196058 and Sg181942 presented similarities with

chromosomes 1 and 3, while the tag Sg180925 aligned with chromosomes 3, 9 and 11. Finally, the tag Sg218368 performed matches with both chromosomes 2 and 11, also here suggesting the occurrence of duplication and translocation events along the genome.

CONCLUSIONS

- The present approach using full length rice reference cDNA sequences permitted the successful identification of 1,460 sugarcane genes associated with the response to pathogen attack.
- The sugarcane transcriptome includes all procured gene categories of KEGG plant-pathogen interaction pathway, unraveling a high abundance of genes associated with HR and SAR, as well as *R* and *PR* genes.
- Most of the identified sequences (74%) presented best matches with *Sorghum bicolor*, followed by *Zea mays* (15%) and *Oryza sativa* (9%) reflecting their taxonomic relationship and also indicating the potential for transferring gene markers from sorghum to sugarcane for mapping purposes.
- Considering the *PR* gene categories identified, most representatives regarded the *PR-9* (peroxidases class), that contributes to plant disease resistance through the deposition of lignin, conferring resistance against a broad spectrum of pathogens. Other classes (*PR-2*, *PR5*, *PR-7*, and *PR-14*) were also well represented, indicating a relative genetic diversity and abundance regarding this category.
- The NBS-LRR *R*-gene class was the most representative, with all families presented matches in sugarcane. Probably the studied sequences represent only part of the diversity and number of *R*-genes that are present in the cultivated sugarcane varieties, especially considering the huge size and redundancy of the sugarcane genome, as compared with most angiosperms, and also the complexity of the epigenetic processes.
- Most of the identified sugarcane sequences matched to rice chromosomes 1 and 3 (133 and 124, respectively), also presenting clustered regions, as expected especially for *R* genes that emerged by duplication events.
- A high number of sequences associated with response to pathogen attack in sugarcane is also active under abiotic stress, especially during drought, confirming observations regarding cross-talk among genes of distinct stress categories. Considering their relative distribution within the rice genome, regions comprising over- and down-expressed SuperSAGE tags are not distributed randomly, presenting consistent co-expression also considering different experimental conditions and stress types.

The identified sequences represent valuable sources for the sugarcane breeding program, allowing their use in biotechnological approaches, with emphasis on transgene experiments. They are also valuable for mapping purposes, especially considering their putative distribution here uncovered when considering available distribution of genes known from the rice genome. Multifunction genes that are important in pathogen response as well as during abiotic stress in tolerant sugarcane cultivars – here identified by the high throughput SuperSAGE assay – are most important candidates for approaches aiming to confer multiple (biotic and abiotic) resistance in sugarcane, a very important strategy considering the actual climate changing scenario.

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