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Comprehensive Analysis of *MeGRAS* Family Genes in Cassava and their Genome Collinearity Relationship with *Arabidopsis* and *Populus* Genome

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Abstract

GRAS transcription factor family genes play diverse role in plant growth and development and often have a role as integrators of multiple development regulatory and environmental signals. Functional characterization and understanding of *GRAS* transcription factor family genes will help to breed, high yielding improved cassava genotypes. In this study, a genome wide analysis led to identification of 78 *MeGRAS* genes in cassava. The genes are distributed in all the chromosomes of cassava except chromosome no. 16. The identified cassava *MeGRAS* family members localized in different sub-cellular compartments including the nucleus, cytoplasm, chloroplast and mitochondria suggest a wider cellular localization and diverse role of *MeGRAS* family members in cassava. Occurrence of tissue-specific (biotic, abiotic, light-responsive, circadian and cell cycle-responsive) /*cis*-regulatory elements in the promoter regions of the *MeGRAS* family showed the potential role of *MeGRAS* family in plant growth, plant development and stress tolerance in cassava. Genome collinearity analysis with *Arabidopsis* and *Populus* genome showed the evolution of the *MeGRAS* family members through duplication and divergence in cassava. This comprehensive analysis contributes for a better understanding of the complexity of *MeGRAS* family members in cassava, and also provide further basis to dissect their potential role in development and stress response of cassava.

Key words: GRAS, transcription factor, genome-wide, cassava, collinearity, *Populus, cis* elements

Introduction

GRAS is one of the important plant-specific transcription factors that regulates various plant developmental process including root and shoot patterning and development, initiation of axillary and shoot meristem and its maintenance, root radial patterning, Gibberellic Acid (GA) signalling, phytochrome A signal transduction (Hirsch and Oldroyd, 2009). The name of these transcription factors was derived after the discovery of first three members: *gibberellic acid insensitive (GAI)*, *repressor of GAI (RGA)* and *scarecrow (SCR)* (Pysh et al., 1999). GRAS family members functions as transcription factor and it plays major regulatory role in modulating the expression of downstream genes in response to various cellular signals. The presence of structural/ functional features, like nuclear localization signals, homopolymeric stretches and two LHR domains proves their function as transcription factors (Liu and Widmer, 2014). *GRAS* transcription factor family genes are well studied and characterised in many crop plants, including *Arabidopsis* (Lee et al., 2008), rice (Tian et al., 2004), Castor Beans (Xu et al., 2016) and *Populus* (Liu and Widmer, 2014). In legumes, GRAS proteins play an important role in root nodulation (Heckmann et al., 2006).

Cassava is an important staple food for 800 million humans in the tropical and sup-tropical regions of the world (Burns et al., 2010). It is a hardy plant and has inherent genetic mechanism to thrive well in the marginal soils, where other crops fail to grow. The starch rich tubers as well as cassava-flour based food products including sago, wafers, papads, etc. are widely used for human consumption (Ravi et al., 1996; Raju et al., 2015). In addition, cassava starch remains as a feed stock for many industries including food, textile, animal feeds, etc. Therefore, identification and functional characterisation of genes would help to develop high yielding cultivars, availability of the high-quality draft genome sequence information of cassava fastens the identification of the genes through comparative genomics approaches (Bredeson et al., 2016). Comparative genomics along with functional genomics approaches have helped to identify candidate genes and gene family members in many crop plants including rice and wheat (Tian et al., 2004; Katiyar et al., 2015; Ravi et al., 2016; Lenka et al., 2019). Many important gene family members namely *MeNBS-LRR*, *MeERF* were identified and characterised in cassava, however no efforts have been made to understand the role of *GRAS* family genes in cassava (Fan et al., 2016; Lozano et al., 2015). Hence, in this study we have done a genome-wide analysis and identified and characterised *MeGRAS* family genes in cassava. This comprehensive analysis would help to understand the complexity and further provide basis for understanding the molecular function of GRAS family in cassava.

Materials and Methods

Genome-wide analysis and identification of *MeGRAS* genes in cassava

A Hidden Markov Model (HMM) profile was built using the sequence information of the *Arabidopsis* and rice *GRAS* amino acid sequences (PF03514). The in-house build GRAS sequenced based HMM was used as query and a profile HMM search was performed against cassava genome (Bredeson et al., 2016; Muthusamy et al., 2016). Structural details including the gene name, sequence id and chromosome location were retrieved from the cassava genome (Bredeson et al., 2016). The exon-intron organisation of *MeGRAS* genes were computed using their gene and CDS sequence information (Muthusamy et al., 2017). Target P 1.1 database used for sub-cellular localization of the *MeGRAS* genes. The SMART tool database was used to identify the conserved domains in *MeGRAS* genes (Letunic and Bork, 2018; Muthusamy et al., 2019). The protein size, molecular weight and isoelectric point (pI) were predicted using expasy tool (https://web.expasy.org/compute_pi/).

Cis-regulatory element analysis

Promoter sequences (putative regions of -1500 bp) upstream to the transcription start site of *MeGRAS* gene were retrieved from the cassava genome database. PlantCARE software was used to predict the conserved *cis*-regulatory regions of the *MeGRAS* gene (Lescot et al., 2002; Muthusamy et al., 2017).

Genome collinearity analysis

The genomes of *Arabidopsis* and *Populus* were downloaded from the NCBI database and the collinearity relationship of *MeGRAS* family members genes with the *GRAS* orthologous of *Arabidopsis* and *Populus* were performed using MCScanX (http://chibba.pgml.uga.edu/mcscan2).

Results and Discussion

Genome-wide analysis and identification of *MeGRAS* family genes in cassava

GRAS transcription factor family genes regulate various cellular process and play an important role in plant growth and development. These family members are well characterised in many crop plants including the model organisms *Arabidopsis* and rice. The wild relative of sweet potato, *Ipomoea trifida* genome encodes 70 *GRAS* family members and these genes were known to play an important role in its growth and development (Chen et al., 2019). Less efforts have been made to understand their role in cassava. In this study, we have used the HMM profile of *GRAS* sequence of *Arabidopsis* and rice as a query and a profile HMM search was performed against cassava genome database to identify the *MeGRAS* gene orthologos from cassava genome database. Our initial, analysis led to the identification of 98 *MeGRAS* family genes in cassava. Further pseudogenes were removed through multiple sequence alignment tool (ClustalW) on the basis of the conserved sequences of the *GRAS* domain (Letunic et al., 2012; Scharf et al., 2001). After removal of pseudogenes, our analysis led to the identification of 78 *MeGRAS* genes in cassava (Table 1). All of the identified MeGRAS proteins were named according to their chromosomal location as described previously (Paul et al.,2016; Sarkar et al., 2009; Waters and Vierling, 1999b; Yu et al., 2016). The structural details, including the gene name, sequence id, chromosome location, protein size, molecular weight, isoelectric point (pI), and sub-cellular localization of the identified *MeGRAS* genes are listed in Table 1. The predicted isoelectric point of MeGRAS proteins ranges from 4.82 to 7.7 (Table 1). The *MeGRAS-11* gene has a maximum isoelectric point of 7.7, whereas *MeGRAS-30* has the lowest pI of 4.82 with an average pI of 5.67 (Table 1). The computed molecular weight of the identified proteins ranges from 26.08 to 132.5 kDa. (Table 1). *MeGRAS-14* gene has a maximum molecular weight of 132.5 kDa, whereas *MeGRAS-72* has the lowest molecular weight 26.08 kDa. (Table 1). Out of 78 *MeGRAS* genes, 51 genes are localized to the nucleus, 14 genes are localized cytoplasm, 11 genes are localized to chloroplast and two genes are localized to mitochondria (Table 1). The SMART (a Simple Modular Architecture Research Tool) online database tool was used for the prediction of conserved domain regions of the MeGRAS proteins. The GRAS domain is present in all the *MeGRAS* genes, conversely, coiled-coil the *MeGRAS* genes was predicted by analyzing the sequence information. The analysis showed that the genes are distributed in all the 18 chromosomes of cassava except chromosome no. 16. Also, all the *MeGRAS* genes are not uniformly distributed among the chromosomes of cassava, however some regions of the genomes are highly enriched with *MeGRAS* genes (Table 1 and Fig. 1). Chromosome 2 encodes maximum of seventeen *MeGRAS*

Table 1. List of the identified *MeGRAS* gene family members in cassava. (aa- amino acids; pI- isoelectric point. Mw- Molecular weight; The subcellular localization of GRAS proteins were predicted through WoLF PSORT and TargetP 1.1 server.)

Gene Name	Loc ID	Gene start(bp)	Gene end (bp)	Strand orientation	pI	Mw(kDa)	Chromosomal localization	Cellular localization
MeGRAS-1	Manes.01G008100.1	1394567	1395907	1	6.01	49.72	01	Cytoplasm
MeGRAS-2	Manes.01G066400.1	19023578	19025551	1	6.06	73.29	01	Chloroplast
MeGRAS-3	Manes.01G134900.1	24977847	24980723	1	4.96	66.24	01	Nucleus
MeGRAS-4	Manes.01G171700.1	27456830	27459618	-1	5.78	74.10	01	Nucleus
MeGRAS-5	Manes.01G171800.1	27461512	27463233	-1	6.30	65.15	01	Cytoplasm
MeGRAS-6	Manes.01G171900.1	27466826	27470370	-1	5.21	87.65	01	Nucleus
MeGRAS-7	Manes.01G172000.1	27472624	27475905	-1	5.51	85.00	01	Nucleus
MeGRAS-8	Manes.01G182400.1	28141683	28144591	1	6.26	49.91	01	Cytoplasm
MeGRAS-9	Manes.02G027000.1	2093804	2096962	1	5.88	74.18	02	Chloroplast
MeGRAS-10	Manes.02G038400.1	2975137	2977534	1	6.10	85.60	02	Nucleus
MeGRAS-11	Manes.02G058800.1	4370590	4371846	-1	7.70	47.21	02	Nucleus
MeGRAS-12	Manes.02G075900.1	5599118	5601415	-1	5.04	67.23	02	Nucleus
MeGRAS-13	Manes.02G093300.1	7003781	7006316	1	4.89	66.50	02	Nucleus
MeGRAS-14	Manes.02G116600.1	8634492	8641352	-1	5.78	132.50	02	Cytoplasm
MeGRAS-15	Manes.02G130800.1	9572625	9575516	-1	6.51	75.47	02	Nucleus
MeGRAS-16	Manes.02G130900.1	9576116	9579293	-1	4.89	65.42	02	Nucleus
MeGRAS-17	Manes.02G131000.1	9580226	9582745	-1	5.93	75.67	02	Nucleus
MeGRAS-18	Manes.02G131100.1	9588875	9590674	-1	7.56	68.05	02	Cytoplasm
MeGRAS-19	Manes.02G142500.1	10549800	10551941	1	5.78	53.05	02	Cytoplasm
MeGRAS-20	Manes.02G161100.1	12157071	12158912	1	5.64	54.25	02	Cytoplasm
MeGRAS-21	Manes.02G173300.1	13639301	13643215	1	4.93	64.65	02	Nucleus
MeGRAS-22	Manes.02G190600.1	15555542	15557177	1	5.88	57.20	02	Nucleus
MeGRAS-23	Manes.02G204600.1	19199375	19201120	-1	4.85	65.74	02	Nucleus
MeGRAS-24	Manes.02G204800.1	19203016	19204740	-1	5.10	65.89	02	Nucleus
MeGRAS-25	Manes.02G212600.1	23544257	23545675	-1	4.91	53.88	02	Cytoplasm
MeGRAS-26	Manes.03G121000.1	21739862	21742872	-1	5.91	62.90	03	Chloroplast
MeGRAS-27	Manes.03G137800.1	23251538	23254386	-1	5.51	59.88	03	Nucleus
MeGRAS-28	Manes.03G203200.1	28375717	28377015	-1	4.89	66.50	03	Nucleus
MeGRAS-29	Manes.03G203700.1	28412678	28414390	1	5.66	61.86	03	Nucleus

MeGRAS-30	Manes.03G203800.1	28416048	28417796	1	4.82	65.14	03	Nucleus
MeGRAS-31	Manes.03G203900.1	28419831	28421582	1	4.89	65.42	03	Nucleus
MeGRAS-32	Manes.03G204000.1	28431180	28432916	1	4.98	64.75	03	Nucleus
MeGRAS-33	Manes.04G145200.1	27074518	27076660	1	5.81	61.59	04	Nucleus
MeGRAS-34	Manes.04G164200.1	28508155	28510978	1	5.84	53.75	04	Cytoplasm
MeGRAS-35	Manes.05G004100.1	316570	318357	-1	6.72	67.92	05	Nucleus
MeGRAS-36	Manes.05G048700.1	3602440	3605481	1	5.42	78.15	05	Nucleus
MeGRAS-37	Manes.05G203600.1	27786398	27787750	1	6.22	50.09	05	Cytoplasm
MeGRAS-38	Manes.06G151400.1	25508652	25511406	1	6.28	69.65	06	Nucleus
MeGRAS-39	Manes.07G117400.1	24573389	24577085	1	5.50	83.54	07	Cytoskeleton
MeGRAS-40	Manes.08G071100.1	11157523	11158851	-1	5.67	48.08	08	Chloroplast
MeGRAS-41	Manes.08G089000.1	22516456	22521479	-1	6.95	86.01	08	Nucleus
MeGRAS-42	Manes.08G095400.1	25006260	25007774	-1	5.11	57.92	08	Nucleus
MeGRAS-43	Manes.08G095500.1	25016503	25017981	1	4.93	55.52	08	Nucleus
MeGRAS-44	Manes.08G165800.1	32640107	32643298	-1	5.61	89.79	08	Nucleus
MeGRAS-45	Manes.09G082000.1	14943024	14947462	1	5.65	61.19	09	Nucleus
MeGRAS-46	Manes.09G109800.1	23307868	23309241	1	5.14	51.83	09	Nucleus
MeGRAS-47	Manes.09G119400.1	24247645	24250729	-1	5.75	76.70	09	Nucleus
MeGRAS-48	Manes.10G029200.1	2451211	2453964	-1	5.93	82.26	10	Nucleus
MeGRAS-49	Manes.10G123300.1	23500784	23502148	-1	5.41	50.32	10	Nucleus
MeGRAS-50	Manes.11G002500.1	291207	293941	1	6.10	53.20	11	Cytoplasm
MeGRAS-51	Manes.11G019900.1	1877962	1880281	-1	5.93	60.93	11	Nucleus
MeGRAS-52	Manes.11G083500.1	11447101	11449150	1	5.58	69.04	11	Chloroplast
MeGRAS-53	Manes.11G125900.1	23537206	23539149	1	5.43	51.50	11	Nucleus
MeGRAS-54	Manes.12G034000.1	2681022	2682847	1	6.08	57.82	12	Nucleus
MeGRAS-55	Manes.12G061900.1	5617544	5620565	1	5.73	59.54	12	Nucleus
MeGRAS-56	Manes.12G110800.1	25676619	25677995	1	5.54	51.10	12	Chloroplast
MeGRAS-57	Manes.12G114100.1	26341990	26344130	1	5.45	56.64	12	Nucleus
MeGRAS-58	Manes.13G013600.1	1360836	1362317	-1	5.88	55.54	13	Cytoplasm
MeGRAS-59	Manes.13G063600.1	6954529	6956960	-1	5.89	61.26	13	Mitochondria
MeGRAS-60	Manes.13G074700.1	9528952	9531310	-1	4.95	49.38	13	Chloroplast
MeGRAS-61	Manes.13G111600.1	23843759	23845647	-1	5.44	56.69	13	Nucleus
MeGRAS-62	Manes.13G113100.1	24005784	24007829	1	5.59	51.84	13	Chloroplast
MeGRAS-63	Manes.13G137100.1	26451459	26453337	-1	5.85	59.80	13	Chloroplast
MeGRAS-64	Manes.14G009600.1	945188	947032	1	5.01	57.60	14	Mitochondria
MeGRAS-65	Manes.14G023500.1	2035177	2037084	-1	6.62	69.47	14	Nucleus
MeGRAS-66	Manes.14G054900.1	4333226	4334797	1	5.84	58.27	14	Nucleus
MeGRAS-67	Manes.15G005100.1	544731	546485	-1	5.30	66.01	15	Nucleus
MeGRAS-68	Manes.15G063500.1	4661624	4666292	1	5.26	59.03	15	Nucleus
MeGRAS-69	Manes.15G078300.1	5674345	5678930	1	5.56	63.34	15	Nucleus
MeGRAS-70	Manes.15G078400.1	5724333	5725933	1	5.95	50.65	15	Nucleus
MeGRAS-71	Manes.15G078700.1	5758208	5760159	1	5.88	56.94	15	Nucleus
MeGRAS-72	Manes.15G078800.1	5781898	5783341	1	6.07	26.08	15	Cytoplasm
MeGRAS-73	Manes.15G130800.1	9943106	9945010	-1	5.54	69.67	15	Nucleus
MeGRAS-74	Manes.17G080200.1	22030159	22032572	-1	5.46	75.30	17	Chloroplast
MeGRAS-75	Manes.18G075300.1	6467564	6469330	1	5.79	54.95	18	Nucleus
MeGRAS-76	Manes.18G085800.1	7410967	7414505	1	4.95	65.10	18	Chloroplast
MeGRAS-77	Manes.18G099600.1	8635069	8636546	1	6.16	52.70	18	Nucleus
MeGRAS-78	Manes.S025700.1	128032	132486	1	5.59	61.26	Scaffold 00731	Nucleus

genes, whereas, chromosome 6 and 7 encodes each one gene (Table 1 and Fig. 1). Three chromosomes viz., 4, 10 and 17 each encodes two *MeGRAS* genes, four chromosomes viz., 5, 9, 14 and 18 each encodes three *MeGRAS* genes, two chromosomes viz., 11 and 12 each encodes four *MeGRAS* genes and two chromosomes viz., 13 and 15 each encodes six *MeGRAS* genes (Table 1 and Fig. 1). Chromosome 1, 3 and 8 encodes, eight, seven and five *MeGRAS* genes, respectively. The gene *MeGRAS-78* is mapped in the Scaffold00731 (Table 1). The chromosomal physical location of the identified *MeGRAS* genes and their orientation details on the chromosome are given in Table. 1. The gene structure of the *MeGRAS* gene family members were studied using the genomic sequence details. The analysis showed that out of 78 genes, 50 *MeGRAS* genes are intron less, 21 *MeGRAS* have two exons. 5 genes have three exons, 1 gene has four exons and 1 gene has nine exons (Fig. 2). Plant *GRAS* family members are known to play diverse role in plant growth and development often has a role as integrators of multiple development, regulatory and environmental signals (Liu and Widmer, 2014). In our study, the *MeGRAS* family members were localized in different sub-cellular compartments including the nucleus, cytoplasm, chloroplast and mitochondria thereby suggesting a wider cellular localization and diverse role of *MeGRAS* family members in cassava (Table 1). Also, the presence of conserved *cis*-regulatory elements (tissue-specific, biotic, abiotic, light-responsive, circadian and cell cycle-responsive) in the promoter regions of the *MeGRAS* genes showed the potential role of *MeGRAS* in plant development and stress tolerance in cassava (Fig. 3). Thus, the *MeGRAS* family members might play an important role in growth and development of cassava and further functional genomics studies would help to identify the important function of *MeGRAS* genes in cassava.

Genome collinearity relationship of *MeGRAS* genes with *Arabidopsis* and *Populus* genome

Genome collinearity analysis shows the distribution and conserved pattern of a set of loci in two different species (Wang et al., 2012). To understand the genome collinearity of *MeGRAS* genes, we have used two different plant genomes viz., *Populus* belonging to same order of cassava (malpighiales) and *Arabidopsis* belonging to

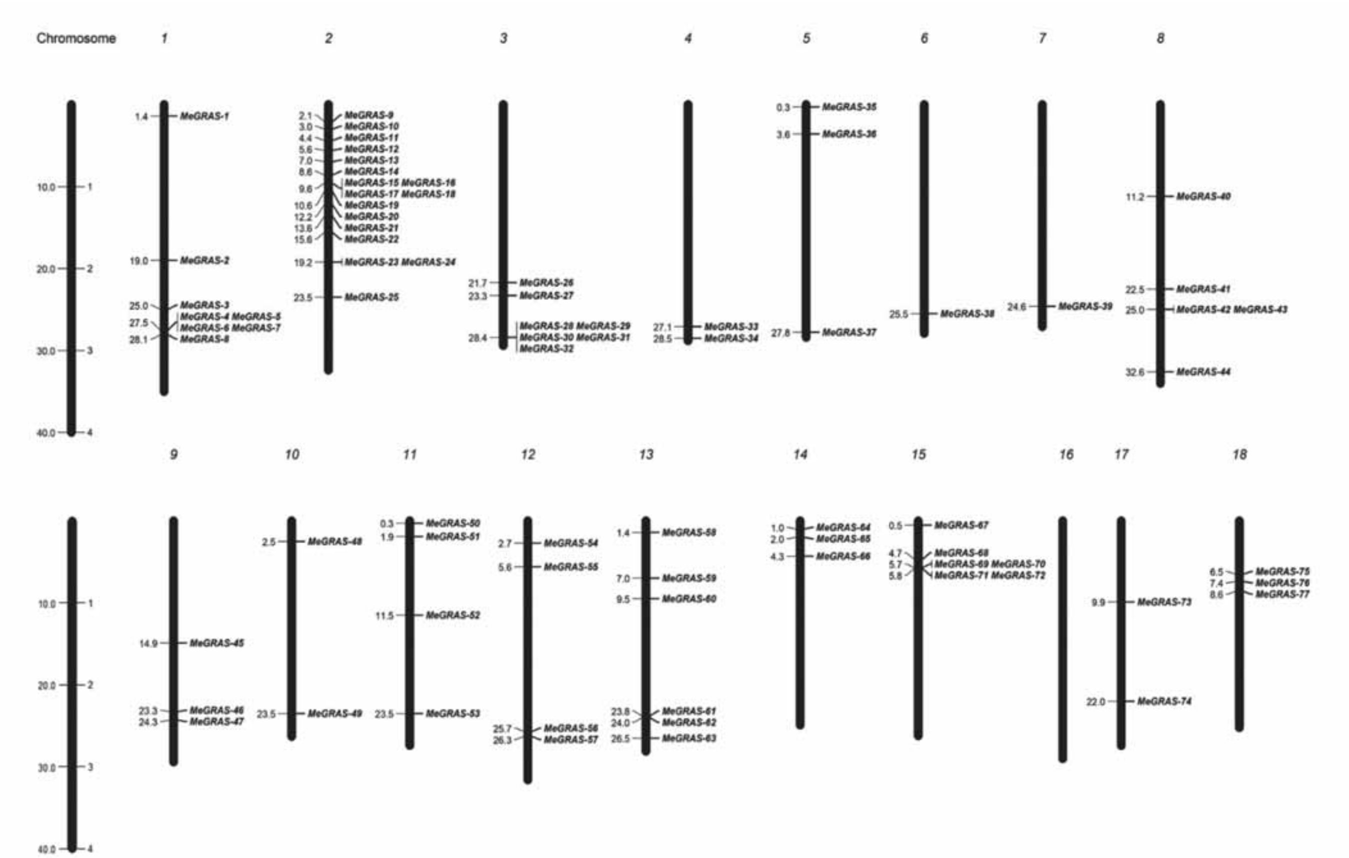

Fig. 1. Chromosomal distribution pattern of *MeGRAS* gene family in cassava

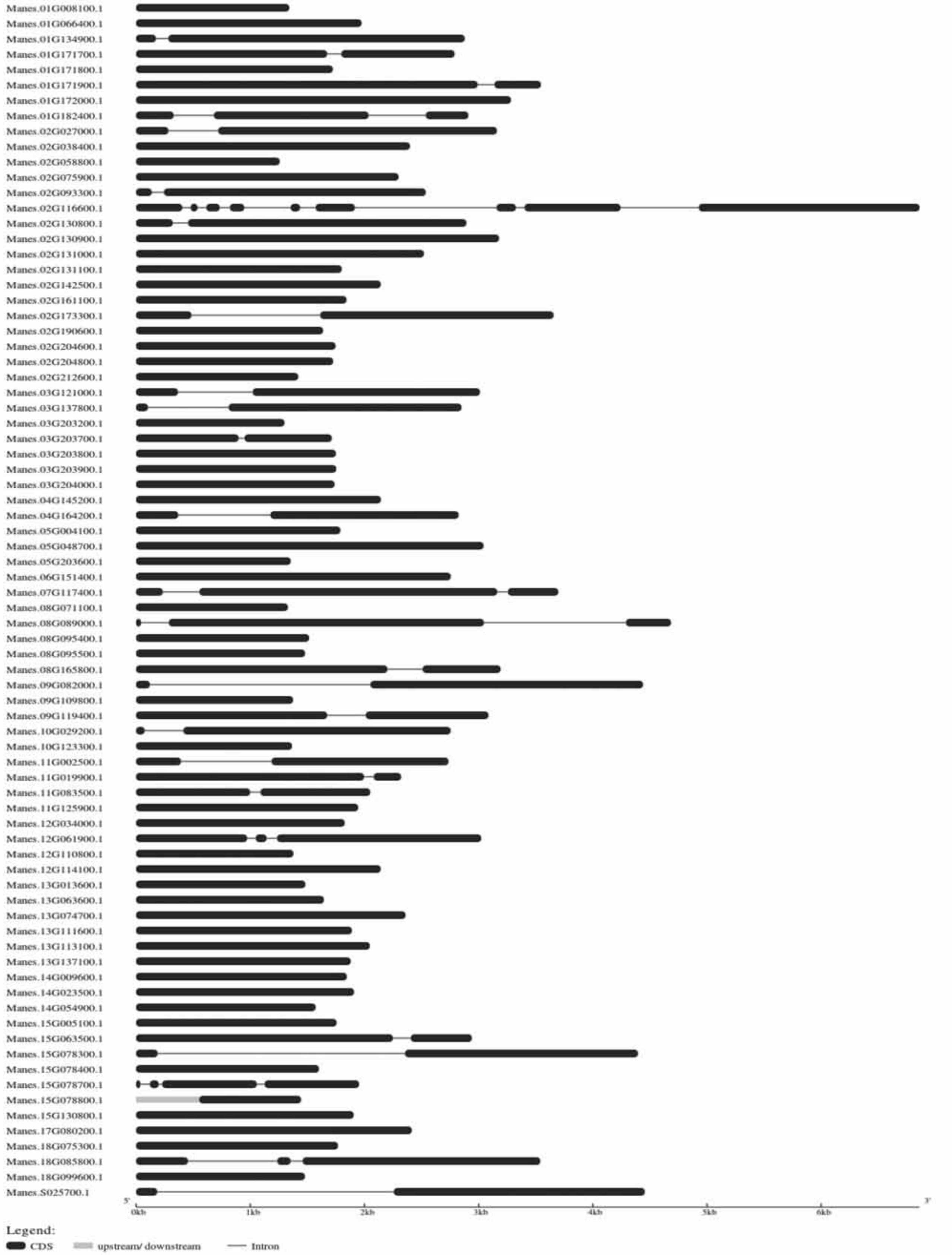

Fig. 2. Exon/Intron organisation of *MeGRAS* family genes in cassava

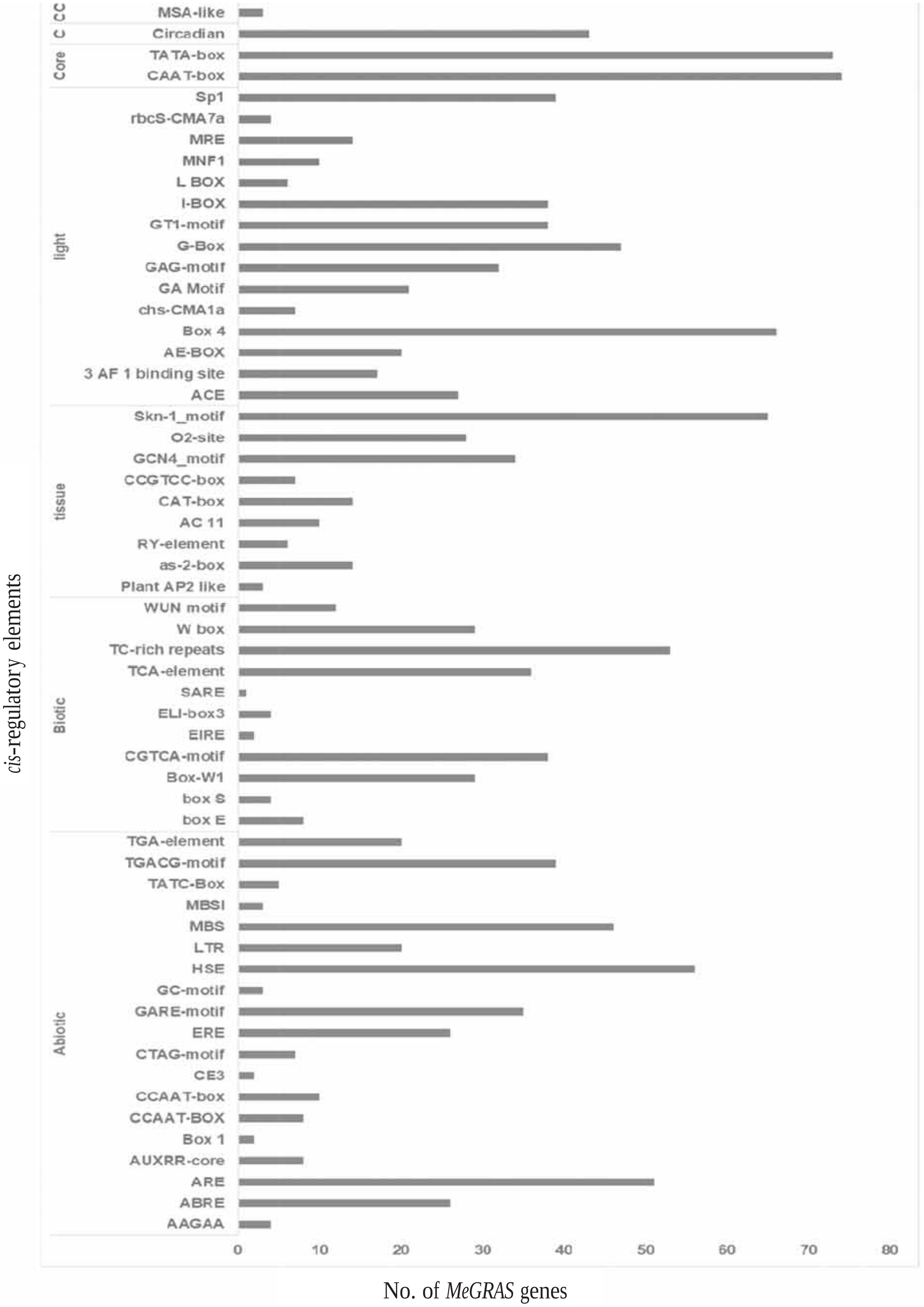

Fig. 3. *Cis*-regulatory elements in the promoters of *MeGRAS* family genes in cassava
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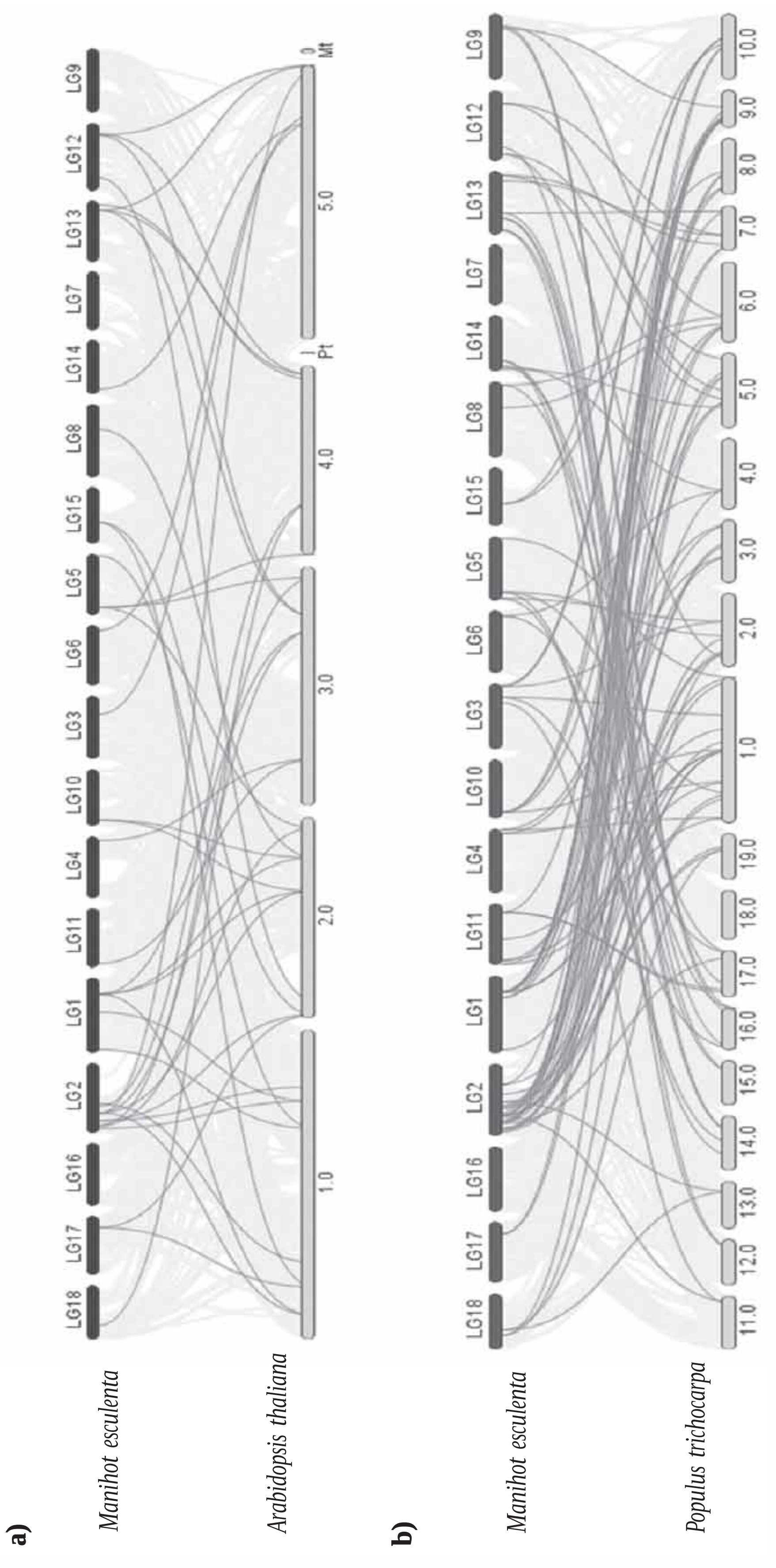

Fig. 4. Genome collinearity relationship of *MeGRAS* family members with plant genomes. a) *Arabidopsis* b) *Populus*. The *MeGRAS* gene family members collinearity relationship with *Arabidopsis* and *Populus* orthologs are shown in the figure. The bar represents the chromosome number and its relative size

different family Brassicaceae. The study showed that 53 members of *MeGRAS* family displayed collinearity with the *Populus* genome, whereas 29 *MeGRAS* family members were displayed collinearity with the *Arabidopsis* genome (Fig. 4). These results showed that the *MeGRAS* family members have evolved through duplication and divergence mechanism in cassava.

Conclusion

Genome-wide analysis revealed that the cassava genome encodes for 78 *MeGRAS* family members. Presence of tissue, biotic and abiotic stress responsive elements in the promoter regions shows the protentional role of *MeGRAS* family members in growth and development of cassava. Genome collinearity analysis of *MeGRAS* family members with *Arabidopsis* and *Populus* genomes revealed that the gene duplication and divergence played an important role in evolution of *MeGRAS* in cassava. Many *GRAS* genes family members have been successfully exploited for improving abiotic stress tolerance in many crop plants. Similarly, *MeGRAS* family members can be exploited for enhancing tolerance to abiotic stresses in cassava. This study contributes to the better understanding of *MeGRAS* family members in cassava and its evolution in cassava. Also, provides further basis to dissect their functional role in growth and development and stress response through functional genomics approaches in cassava.

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