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Full Length Research Paper

Recent advances in cowpea [*Vigna unguiculata* (L.) Walp.] "omics" research for genetic improvement

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After decades of research on cowpea, significant amount of omics datasets are available and useful in understanding the genetic relationship between *Vigna unguiculata* ssp. *unguiculata* and other species belonging to the same genus as well as its genetic variation. Besides, the development of genetic map allowed the chromosome localization of molecular markers associated with disease resistance, seed weight, dehydrin, drought-induced genes, maturity and earliness, and the recent progresses made on cowpea genomic resources development and the availability of a genetic transformation protocol increased the chance to identify more genes and to study their expression. In addition, transcriptomic datasets suggested that many genes are expressed during drought, heating or in nitrogen deficiency conditions as well as during symbiosis and iron storage. Proteomic and metabolomic analyses revealed that the protein and metabolite fractions specifically accumulated in the embryogenic cell suspension and in manganese toxicity conditions, respectively. However, the integration of all these information will promote the improvement of cowpea production.

Key words: *Vigna unguiculata* ssp. *unguiculata*, cowpea, genomic, transcriptomic, proteomic, metabolomic.

INTRODUCTION

The increasing agricultural production became an urgent issue since projections suggest that the global population will reach 9 billion people by the middle of this century (Godfray et al., 2010). According to the estimation, 1 billion people will suffer from hunger because they do not have access to food in terms of quantity (protein deficit) and quality (micronutrient deficit), while the vast majority will be living in the developing countries. Besides the increase of the human population, the world is facing new challenges, such as shrinking cultivable lands, stagnant yields, increasing biofuel demands, new emerging pathogens and pests, and salinity and flooding due to climate change. It has become a priority for plant biologists to deliver new improved genotypes that can feed the growing population and that are adapted to the new environmental conditions. Such challenges should be overcome in the age of "omic" since the whole genomes of many important agricultural crops have been sequenced or are under way (Mochida and Shinozaki, 2010). "Omics" approaches will enhance our understanding of gene function, regulatory networks occurring in stress conditions, development and growth in association with phenotypic change in many important plants. In addition, the development of omic resources in cowpea will be promoted by its relatively small genome estimated to be 620 Mbp (Arumuganathan and Earle, 1991; Takeda and Matsuoka, 2008).

Cowpea [*Vigna unguicalata* (L.) Walp.] is a diploid species (2n = 2x = 22) belonging to the section catiang, subspecies *unguiculata*, genus *Vigna*, tribe *Phaseoleae* and the family *Fabaceae* (Maréchal et al., 1978). It is a pantropical herbaceous nitrogen fixing plant known by a variety of names, but cowpea is the most popular

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Abbreviations: PODs, Apoplastic peroxidases; **EST,** expressed sequences tag; **SSH,** suppression subtractive hybridization; **ABA,** abscisic acid; **CPRD,** cowpea clones responsive to dehydration; **TFs,** transcription factors; **TAFs,** transcription associated factors; **GSRs,** gene-space sequence reads; **MF,** methylation filtration; **QTL,** quantitative trait loci; **ISSR,** intersimple sequence repeat; **RAPD,** radom amplified polymorphic DNA; **SAMPL,** selectively amplified microsatellite polymorphic locus; **AFLP,** amplified fragment length polymorphism; **ITS,** intergenic sequence.

worldwide name. In the United States, it is called black-eyed beans, black-eyed peas or southern peas, whereas in Indian and Brazil, it is referred to as lobia and caupi, respectively. In French speaking countries of Africa, Niébé is the common name, but there are local names depending on the ethnical groups, such as 'niao' in Senegal, 'wake' in Nigeria and 'luba hilu' in the Sudan. Several parts of cowpea like dried or fresh seeds, leaves, fresh immature pods and roots are used in human consumption and animal feeding. The seed protein contents range from 23 to 32% of seed weight rich in lysine and tryptophan, and a substantial amount of mineral and vitamins (folic acid and vitamin B) necessary for preventing birth defect during the pregnancy stage (Nielson et al., 1993; Hall et al., 2003). Cowpea is known also as containing a low amount of fat and high level of fiber which can prevent heart disease by reducing the low-density lipoprotein (Phillips et al., 2003). In addition, cowpea consumption increases glucose blood more slowly because of the slowly digestibility of the legume starch promoting its usage for diabetics (Phillips et al., 2003). Besides its nutritional value, cowpea feeds millions of people in the developing world with an annual worldwide production estimated around 4.5 million metric tons on 12 to 14 million ha. The drier savanna and the Sahelian region of West and Central Africa produce about 70% of cowpea's worldwide production, with Nigeria, Niger and Brazil being the largest producers (Singh et al., 2002). This production is mainly limited by a wide range of biotic constraints like virus (Cowpea aphid-borne mosaic virus, CABMV), bacteria (*Xanthomonas campestris pv vignicola*), fungus (*Choanephora spp.)*, insects (*Aphis craccivora*, *Megalurothrips sjostedti*, *Callosobruchus maculatus,* etc.) plants (*Striga gesnerioides* and *Alectra vogelii*) and nematodes (*Meloidogyne incognita),* and also by abiotic constraints (Singh, 2005).

This review provides an overview of recent advances carried out in cowpea omic researches during the past decades. It will focus particularly on genomic, transcriptomic, proteomic and metabolomic findings and how these datasets can be integrated for the crop's improvement.

WHAT DO WE KNOW OF GENOMIC?

Comparative genomic

Comparative genomic studies on cowpea were based on chloroplast or nuclear genome analyses. Studies based on chloroplast genome restriction performed by Vaillancourt and Weeden (1992) supported a limited gene flow between the cultivated form and the wild subspecies *V. unguiculata* ssp *dekindtiana*. The wild accession possessed a plastome type similar with the one found in the cultivated form, suggesting that *dekindtiana sensu* Verdcourt is its progenitor. These findings were confirmed later with studies based on the intergenic sequence (ITS) nuclear DNA and the chloroplast DNA *TrnLF* sequencing (Goel et al., 2002; Diouf et al., unpublished data). Furthermore, the controversial center of domestication event of cowpea is now recognized as occurring in the northern part of Africa (Coulibaly et al., 2002).

Structural genomic

The recent influx of molecular markers has enhanced our understanding of cowpea's genome structure and organization. Studies based on RAPD, DAF and SSR markers revealed a low genetic diversity among cowpea varieties and molecular polymorphism between drought tolerance and sensitive varieties, and also between the higher and lower nitrogen fixing cowpea accessions (Spencer et al., 2000; Li et al., 2001; Tosti and Negri, 2002; Fall et al., 2003; Badiane et al., 2004; Diouf and Hilu, 2005). Using amplified fragment length polymorphism (AFLP) and selectively amplified microsatellite polymorphic locus (SAMPL) markers, Tosti and Negri (2005) analyzed the genetic variation within and among three neighbouring cowpea landraces currently cultivated in central Italy. This investigation showed a high genetic diversity within landraces induced by drift, landraces isolation, farmer selection and migration within the landrace. This finding could be sustained by uncontrolled gene flow as suggested by Nkongolo (2003) who reported similar results by studying Malawian cowpea landraces with radom amplified polymorphic DNA (RAPD) technology. Other authors reported that intersimple sequence repeat (ISSR) gave higher level of polymorphism in cowpea than RAPD markers between landraces from different regions of Algeria (Ghalmi et al., 2010). This technology showed that *V. triphylla* and *V. reticulata* were the most closely related species to *V. unguiculata* and *V. vexillata* (Ajibade et al., 2000; Goel et al., 2002). This basic information is useful for the successful transfer of the agronomically important traits in the cultivated cowpea, as these species are known to be resistant to many diseases affecting cowpea.

Recently, Fang et al. (2007), using AFLP markers on six cowpea breeding programs from West Africa and USA, and 27 landrace accessions from Africa, Asia and South America, reported that they shared a minimum of 86% genetic similarity. They showed that the accessions from Asia and USA were clustered together suggesting that they have the same origin outside West Africa, while the ones from Africa and Brazil were closer. These results suggested the European origin of US' cowpea, but suggested that Brazilian's cowpea came from Africa (Fang et al., 2007; Perrino et al., 1993). In their conclusions, the authors recommended the implantation of the breeding program between USA, Asia and West

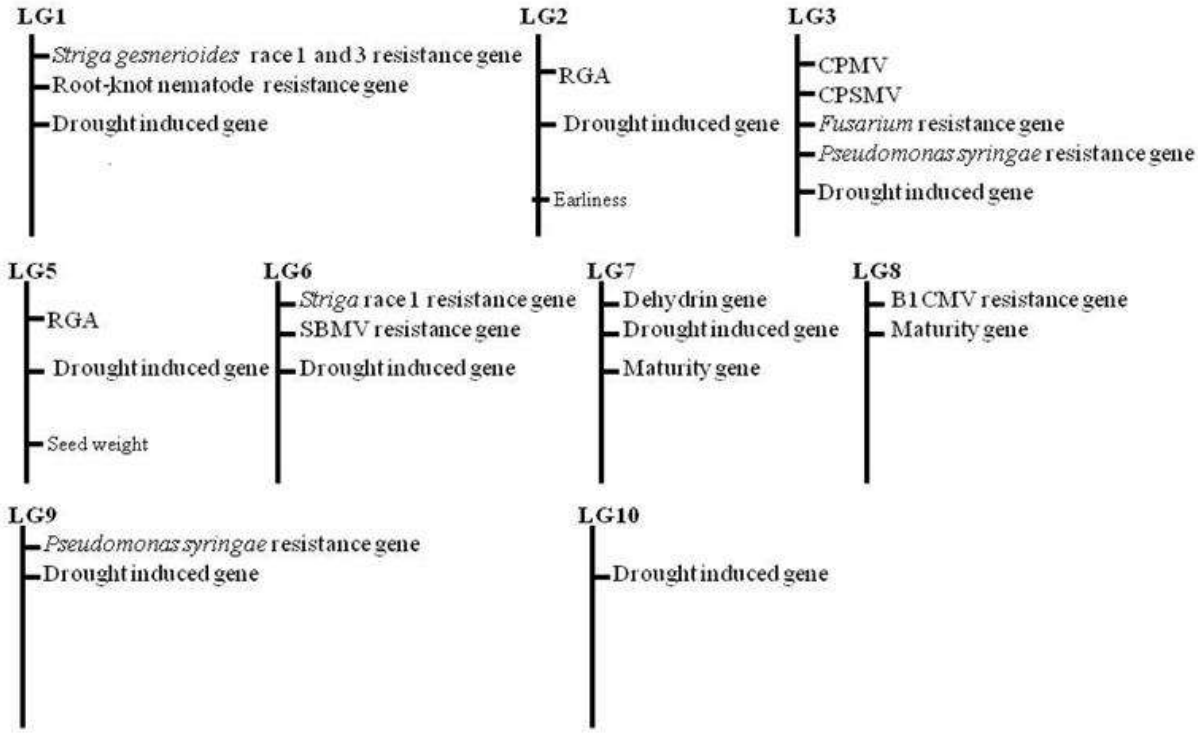

Figure 1. A summary of the schematic representation of the linkage map of cowpea. BICMV: Blackeye cowpea mosaic potyvirus; CVMV: cowpea mosaic virus; CPSMV: cowpea severe mosaic virus; LG: linkage group; RGA: resistance gene analogs; SBMV: southern bean mosaic virus. LG4 and LG11 are not represented because no significant marker was identified.

Africa cowpea, and an introgression between West Africa with Asia and other germplasm from other parts of Africa for improvement.

The first attempt to build a genetic map of cowpea was performed by Fatokun et al. (1992) by using a population resulting from a cross between an improved genotype and its wild progenitor *V. unguiculata* ssp*. dekindtiana*. Despite the disadvantage of this type of cross, which may be related to the identification of the loci that may be polymorphic only between more divergent genotypes, but not between more closely related genotypes, especially the ones of interest, the authors located a quantitative trait loci (QTL) for seed weight. This QTL was conserved beween cowpea and *V. radiata* ssp *sublobota*.

The second linkage genetic map developed on cowpea consisted of 181 loci including 3 morphological markers, and a biochemical marker (dehydrin) allowed to map genes involved in earliness and seed weight respectively in linkage group 2 and 5 (Menéndez et al., 1997). The third map was developed on populations resulting from different crosses between lines resistant to different races of *S. gesnerioides* with susceptible lines. According to their conclusions, the markers linked to *S. gesnerioides* races 1 and 3 are located on the linkage group 1 (LG) of the cowpea genetic map (Ouédraogo et al., 2001). The fourth map showed that the markers linked to the *S. gesnerioides* race 1 and 3 resistance genes, and resistance to several root-knot nematodes are located on the LG1. In contrast, on the LG6 was mapped the markers linked to the *S. gesnerioides* race 1 (Figure 1). The markers linked to Black eye cowpea mosaic potyvirus (BlCMV) is on the top of LG8, whereas southern bean mosaic virus (SBMV) marker is on LG6. Cowpea mosaic virus (CPMV), cowpea severe mosaic virus (CPSMV) and *Fusarium* resistance genes are all mapped on LG3. However, the resistance gene analogs (RGA) are mapped on LG2 and LG5. The markers linked to the resistance of *Pseudomonas syringae* were mapped on LG9 and LG3, while the dehydrin gene previously located on LG1 was mapped on LG7 (Ouédraogo et al., 2002a; 2002b). Other investigators reported that the QTL associated with the drought induced was on LG1, 2, 3, 5, 6, 7, 9 and 10, but the one for maturity was on LG7 and 8 (Muchero et al., 2009a). Recently, the identification of the single feature polymorphism (SFP) of *Glycine max* and its use to build a consensus genetic map of cowpea has led to the estimation of the genome structure based on synteny analysis. The analysis revealed a high macrosynteny between *G. max* and cowpea, as well as between cowpea and *Medicago trunculata*. The LG 5 and

Table 1: Key dates of cowpea genetic transformation

Date	Gene	Technique for DNA delivery	Result	Reference
1986	*Kanamycin resistant*	*Agbacterium tumefaciens*	Transgenic calli	Garcia et al., 1986
1987	*Kanamycin resistant*	*Agrobacterium tumefaciens*	Transgenic calli	Garcia et al., 1987
1991	*gus*	*Agrobacterium tumefaciens*	Putative transgenic plant	Penza et al., 1991
1992	*gus*	Biolistic	Transient expression	Penza et al., 1992
1993	*gus*	Biolistic	Transient expression	Akella and Lurquin, 1993
1996	*hygromycin-resistant*	*Agrobacterium tumefaciens*	Transgenic plant No evidence of transgenic Progenies	Muthukumar et al., 1996
2003	*bar*	Biolistic	Small proportion of ransgenic progenies	Ikea et al., 2003
2006	*bar*	*Agrobacterium tumefaciens*	Transgenic progenies	Popelka et al., 2006
2007	*Kanamycin resistant*	*Agrobacterium tumefaciens*	Transgenic progenies	Chaudhury et al., 2007
2008	*ahas*	Biolistic	Transgenic progenies	Ivo et al., 2008
2008	*α-amylase inhibitor-1*	*Agrobacterium tumefaciens*	Transgenic progenies	Solleti et al., 2008

7 of cowpea encompass a lot of regions from *G. max* chromosomes 2, 14 and 17, and chromosomes 5 of *M. trunculata,* respectively. Consequently, cowpea showed microsynteny with *G. max*, *M. trunculata* and *Arabidopsis* (Muchero et al., 2009b).

On the basis of the sequencing and analysis of the hypomethylated portion of cowpea genome, selectively cloned by methylation filtration (MF) technology, 250,000 gene-space sequence reads (GSRs) were generated to represent 160 MB (25.86% of the cowpea genome). Some of them are involved in the catalytic activity and metabolic processes or genes encoding transcription factors (TFs) and transcription associated factors (TAFs) (Timko et al., 2008). As such, this technology allowed the full-length isolation of the gene controlling *S. gesnerioides* resistance race 3 (Li and Timko, 2009).

Functional genomic

The first attempts carried out by Garcia et al. (1986, 1987) and Penza et al. (1991) demonstrated that cowpea is susceptible to *Agrobacterium tumefaciens*, and that a stable expressed *kanamycin* resitance gene can be obtained, but their protocol failed to generate transgenic plants (Table 1). To overcome this constraint, direct foreign DNA delivery into zygotic embryo cells was experienced by several investigators (Penza et al., 1992; Akella and Lurquin, 1993). Three years after people were back to indirect DNA transfer and transgenic cowpea, expressing *hygromycin-resistant* gene was produced by co-cultivating detached cotyledonary explants with *A. tumefaciens,* but there was no evidence of their seeds germination and transmission of the transgenes on the progenies according to Mendelian laws (Muthukumar et al., 1996). In 2003, with particles bombardment technique of embryonic axes use, putative transgenic cowpea was obtained, but no evidence of transgene stability was demonstrated (Ike et al., 2003). Three years later, the first success of obtaining transgenic cowpea, expressing the *bar* gene and transmitting the transgene in the progenies in the Mendelian fashion was described despite the weakness of the frequency of the transformation event (0.05 - 0.15%) (Popelka et al., 2006). This technique was improved by co-cultivating cotyledonary node explants with *A. tumefaciens* leading to a transformation efficiency at 0.76% and the inheritance of the transgene in the offspring (Chaudhury et al., 2007). Recently, Ivo et al. (2008), using biolistic methods, were able to express the *herbicide imazapyr* and the *gus* genes under the control of *ahas5 act2* promoter, respectively. However, the first and second generation of transgenic plants were expressed by *gus*

and *aha* genes. All these efforts allow the development of transgenic cowpea expressing *α-amylase inhibitor-1* (*αAI-1*) gene under the control of *bean phytohemagglutinin* promoter, in which their seeds' progenies strongly inhibited the development of *C. maculatus* and *C. chinensis* (Solleti et al., 2008).

WHAT DOES TRANSCRIPTOMIC TELLS US?

Cowpea is known to have a better tolerance to drought and high temperature compared to other legumes (Hall, 2004). Therefore, it is important to understand the mechanisms developed by cowpea in these conditions before implementing a breeding program. Transcriptomic approaches suggested that several strategies are developed by cowpea for preventing lipids and proteins degradation, and generation of reaction oxygen species (ROS) like superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^·$). Preserving membrane integrity by avoiding membrane proteins degradation is essential for plants to survive in drought stress. Several authors reported that, this strategy was developed by drought tolerant cowpea cultivars by maintaining the level of expression of certain genes such as *cystatin* and *aspartic protease*, promoting membrane integrity. The transcripts coding these proteins respectively (named *VuC1* and *VuAP1*) were isolated in drought tolerant cowpea cultivars subjected to water deficit and their expression localized in different organs (de Carvalho et al., 2001; Diop et al., 2004). Also, the second important constituent in the membrane structure known as lipid needs to be preserved during drought stress induction. The investigators reported that, the expression of the gene encoding phospholipase D1 (*VuPLD1*) was moderately increased in the drought tolerant cowpea cultivars (Maarouf et al., 1999), in that phospholipase D is a major lipid-degrading enzyme in plants sensitive to drought. In contrast, the phosphatidic acid phosphatase accumulated in different organs of cowpea, could be a molecular signal involved in lipid membranes modification, probably by interacting with phospholipase D1. As such, several putative regulatory elements might be located in this promoter region (Marcel et al., 2000; França et al., 2008). Membrane integrity include also the chloroplast envelope and thylakoid membranes where the main component's [digalactosyl-diacylglycerol (DGDG)] biosynthesis is stimulated under water stress of the tolerant cultivars resulting from a high expression of this gene (Torres-Franklin et al., 2007). Another challenge that needs to be addressed is related to the reaction of oxygen species (ROS) which causes oxidative damage of many cellular components including lipids, proteins and nucleic acids (Haliwell and Gutteridge, 1986). Now, it is widely admitted that ROS formation in plant cells was lowered by alternative oxidase (Aox) activities. The alternative oxidase (Aox) 2b of *V. unguiculata* (*VuAox2b*) is over-expressed in osmotic stress induced by polyethylene glycol (PEG), but under-expressed in salt stress conditions in tolerant cultivars (Costa et al., 2007). Bioinformatic analysis suggests that cis-regulatory elements exist in the promoter regions (Costa et al., 2010). Water deficit stress induces hydrogen peroxide (H_2O_2) formation causing serious damage in plant cells, while its detoxification becomes an imperious necessity for survival. The cowpea tolerant cultivars over-express the gene that encodes ascorbate peroxidase in the chloroplast, while this enzyme is activated in the cytoplasm, peroxisome and chloroplast (d'Arcy-Lameta et al., 2006).

Other investigators showed that several transcripts known as CPRD (cowpea clones responsive to dehydration), CPRD8, CPRD14, CPRD22 and *VuNCED1* encode a 9-cisepoxycarotenoid dioxygenase responsible for ABA (abscisic acid) biosynthesis during drought, high salinity and heat stresses that are highly expressed (Iuchi et al., 1996; 2000). Recently, uncharacterized genes which are down-regulated in drought conditions were reported by Coetzer et al. (2010) by using suppression subtractive hybridization (SSH).

In heat stress conditions, analysis of transcripts expression showed 600 bands, among which 55 and 9 were up-regulated and repressed, respectively (Simoes-Araùjo et al., 2002). However, these transcripts showed homologies with low molecular weight heat shock proteins, wound-induced proteins, disease resistance protein, xylan endohydrolase isoenzyme and different housekeeping genes. On the contrary, in cold conditions, cowpea seedling expresses a lipid transfer protein in their leave tissues, as well as during fungal infection where it plays an antimicrobial function against pathogens (Carvalho et al., 2006).

In other conditions such as in nitrogen deficiency, a decrease of *pur5* transcript level which codes aminoimidazole ribonucleotide synthetase involved in purine synthesis was noticed in cowpea, while the amount of the transcript *pur3* was relatively stable (Smith et al., 2002); In addition,in symbiotic association with *Rhizobium, the gene encoding for the leghaemoglobin (lbII), a gene similar to the soybean leghaemoglobin lbII was abundantly expressed (Arredondo-Peter et al., 1997)*. In legumes and actinorhizal plants, hemoglobin is known to transport oxygen into the infected zone of the nodule, thereby decreasing its partial pressure and preserving nitrogenase activities (Franche et al., 1998).

In addition, the transcript of the genes that was coded for three different plants (ferritin protein) responsible for iron transport and storage within cells was found in the developing leaves and roots of cowpea in normal conditions (Wicks and Entsch, 1993; Wardrop et al., 1999). Presently, the availability of 187,660 expressed sequences Tag (EST) of cowpea resources at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/nucest?term=vigna%20ungu

iculata) has led to the characterization and validation of 102 SSR markers, among which 64.7% have significant homology with identified proteins. Despite the fact that about 31.7% of the SSR were located in the coding sequences, their transferability on others *Vigna* species was strongly demonstrated (Gupta and Gopalakrishma, 2010).

WHAT ABOUT PROTEOMIC?

The analysis of proteome composition of the embryogenic cell suspensions led to the resolution of 550 proteins, among which 128 were isolated for trypsin digestion. Sixty seven different proteins involved in many biological processes like metabolism, hormone response, cell growth-division, transport, cytoskeleton composition, protein synthesis and processing, regulation and signal transduction, disease, defense and stress response were identified. The most abundant among these are chitinase and ribonuclease belonging to the family of PR-4 and PR-10 proteins, respectively (Nogueira et al., 2007). Also, a wide range of proteins were synthesized during manganese toxicity such as acidic apoplastic peroxidases (PODs) and pathogenesis-related proteins like glucanase, chitinase and thaumatin-like proteins (Fecht-Christoffers et al., 2003).

IS METABOLOMIC DRAGGING ITS FEET BEHIND?

To date, the only paper referring to metabolome assessment on cowpea, found in the databases is related to the effect of manganese (Mn) toxicity on metabolite accumulation, such as ferulic acid, which appeared to be down-regulated in Mn-sensitive and up-regulated in Mn-cowpea tolerant leaf tissue. Also, the Mn toxicity affects the amount of other metabolites in cowpea (Führs et al., 2009).

FUTURE DIRECTIONS

Nowadays, the great progress observed on cowpea genome sequencing (Timko et al., 2008), in combination with the availability of “omic” resources from model legumes (sequenced and annoted genome) and bioinformatic tools, should make possible to identify more quickly, genes that govern the agronomically important traits by using synteny approaches. The regulation of the expression of these genes will be studied using genetic transformation protocol developed on cowpea. After ending the sequencing and the annotation of cowpea genome, more efforts need to be done to understand the interactions between the small non coding RNA (small interfering RNA, micro RNA, trans-acting RNA, etc) known to play a role in *Arabidopsis* (Borsani et al., 2005) and their targets, the signaling pathways involved in many biological processes, the role of putative transposable elements and the putative co-evolution between cowpea and its pests and parasites. There is need for more studies to be done on cowpea proteome, metabolome, lipidome, ionome analyses and the usefulness exploration of the integration of phenomic approaches. As such, the handling and integration of all these massive omics datasets will lead to the implementation of a totalomic platform (Toyoda and Wada, 2004) for a comprehensive study of all the systems. Conclusively, totalomic platform will require more computer scientists, mathematicians and statisticians, as well as experts in biology, in order to improve cowpea production and the quality of its products.

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