



Finger Millet [*Eleusine coracana* (L.) Gaertn]: An Orphan Crop With a Potential to Alleviate the Calcium Deficiency in the Semi-arid Tropics of Asia and Africa

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Finger millet plays a vital role in the food and nutritional security of many people in developing countries particularly in Asia and Africa. It is a staple food for poor people in many regions of Asian (India, China, Nepal, and Sri Lanka etc.) and African (South Africa, Ethiopia, Kenya, Uganda, and Nigeria etc.) countries. Finger millet contains nutrient rich components such as dietary fibers, minerals, vitamins, and phytochemicals that include phenolic compounds with several potential health benefits. Calcium (Ca) is an important macronutrient for healthy life of plants, humans and animals. It plays an indispensable role in structure and signaling and its deficiency causes low bone density, osteoporosis, colon cancer etc. Finger millet grains contain exceptionally higher amount of Ca (>300 mg/100g) when compared to other major cereals. Ca transporter and sensor family genes are involved in the uptake, transport and accumulation of Ca. Understanding the molecular mechanisms of Ca transporter and sensor family genes is important for growth, development and seed fortification in finger millet. Expression analysis of Ca transporter and sensor family genes has been carried out in various tissues of finger millet. Only a very little research work has been done to understand the Ca accumulation in the grains of finger millet. In this review, we discuss the nutritional importance and health benefits of finger millet. We discuss the studies on Ca sensor, accumulation and transport genes that help to improve the grains of finger millet with special reference to Ca. Improved Ca content in finger millet may help to alleviate the Ca deficiency throughout the world particularly in the semi-arid tropics of Asia and Africa.

Keywords: calcium deficiency, calcium transporters, finger millet, genome-wide association studies, health benefits, quantitative trait loci

INTRODUCTION

Food security remains a major challenge especially in less developed countries. Orphan crops like cereals, legumes, vegetables and tubers play a vital role in the food security and livelihood of resource poor farmers and consumers in the developing countries of Asia and Africa (Tadele, 2019). Cereals including rice, wheat, maize, sorghum and millets are rich sources of nutrients for

humans and animals in these countries. Finger millet (*Eleusine coracana*) is a nutrient rich crop. Finger millet is being used as food (grains) in developing countries and as animal feed (straw) in developed countries indicating that it is considered as a poor man's food (Ceasar et al., 2018; Wambi et al., 2020). It is a major crop in the arid and semiarid regions of developing countries of Asia and Africa (Ceasar et al., 2018; Krishna et al., 2018, 2020). Among the various millets, finger millet ranks fourth on a global scale of production next to sorghum (*Sorghum bicolor*), pearl millet [*Cenchrus americanus* (new name), *Pennisetum glaucum* (old name)] and foxtail millet (*Setaria italica*) (Upadhyaya et al., 2007; Maharajan et al., 2019). Millets are also nutritionally superior to rice and wheat since their grains contain high amounts of vitamins, iron, carbohydrate, calcium (Ca), potassium, zinc, phosphorus, magnesium, and essential amino acids (Saleh et al., 2013).

Ca is required for a number of basic regulatory functions such as transmission of nerve impulses, contraction and relaxation of muscles, blood coagulation cascade, activation of enzymes, stimulation of hormonal secretion and so on in human body and so on (Pravina et al., 2013). It also helps in the protection from cancers including colorectal, ovarian, breast, and prostate cancers (Goodman et al., 2002; Gao et al., 2005; Lin et al., 2007). Intake of Ca is important for development of fetal skeleton, increasing birth weight prenatal hypertension and avoidance of pre-eclampsia in pregnant women (Chan et al., 2006; Puranik et al., 2017). Around the world, 3.5 billion people are affected by Ca deficiency, with ~90% of people affected in Africa and Asia (Kumssa et al., 2015). Ca deficiency is a serious health problem both in the developing countries of Asia and Africa (Sharma et al., 2017). Human diets with low levels of Ca has been linked to diseases such as osteoporosis and indirectly to rickets (through Vitamin D) (Heaney, 1993; Chan et al., 2006; Pettifor, 2008). Such diets with low Ca levels is prevalent among 80% of elderly population and are consequently affected by osteoporosis worldwide (Bhatia, 2008; Pettifor, 2008). Elderly population is mostly affected by Ca deficiency predominantly in the form of osteoporosis and osteopenia (Puranik et al., 2017). The onset of bone decalcification and demineralization leads to reductions in bone mass causing the osteoporosis among above 50 years old men and menopausal women (Michaelsson et al., 2005). In particular, 80% of the women suffer from osteoporosis (Sharma et al., 2017). Therefore, osteoporosis is a major problem in developing countries (Sozen et al., 2017). The World Health Organization (WHO) considered that osteoporosis as the next main public healthcare concern globally, after cardiovascular diseases afflicting almost 75 million people in Europe, the United States of America and Japan. Hypocalcaemia disease occurs in human body when Ca levels are low in the blood (Fong and Khan, 2012). Milk as well as dairy products are one of the major sources of Ca in human diets. If people

consume 1 L of milk per day they will get the recommended amount 1000–1300 mg of Ca (Thorning et al., 2016). Most poor people living in the developing countries of Asia and Africa cannot afford to buy and consume 1 L of milk per day (Puranik et al., 2017). These people consume <500 mL of milk per day. Unavailability of recommended amount of milk among poor population and lactose intolerance lead to Ca deficiency (Curry, 2013).

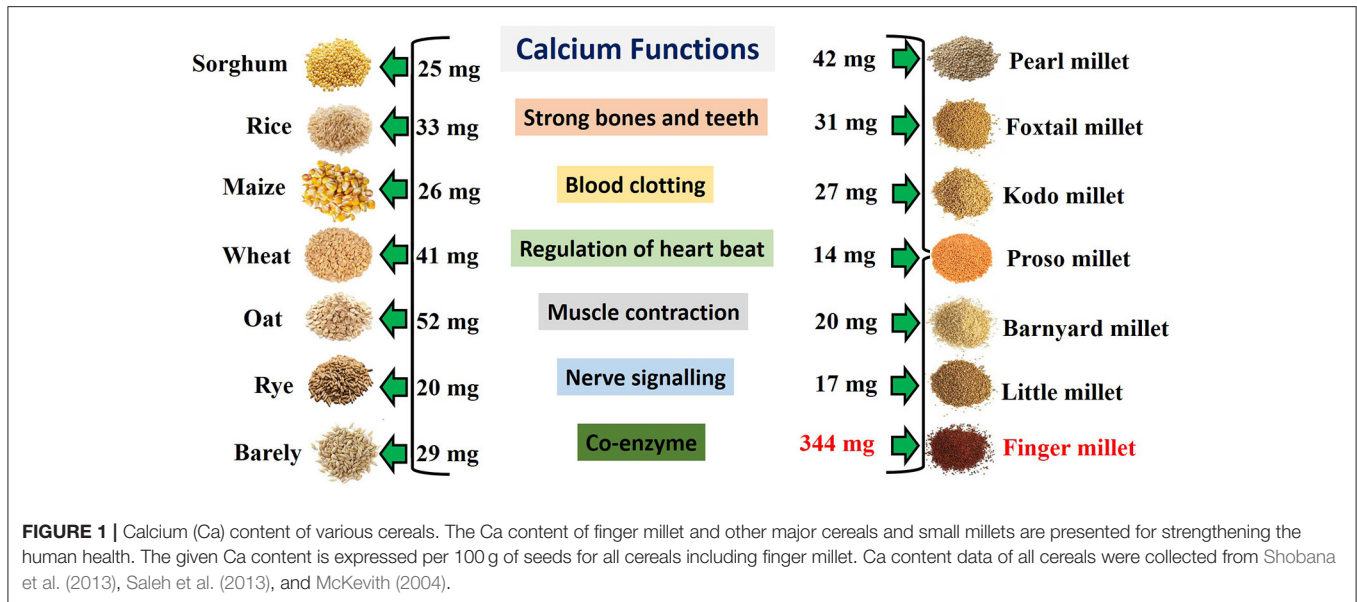
Plants are the cheap, convenient and alternative source of dietary Ca. People frequently consume cereal based food products which are low in Ca. Major cereal crops may not provide adequate amount of Ca for low income people. Finger millet contains higher amount of Ca compared to the other major cereals (Figure 1). For example, the Ca content in finger millet (344 mg/100 g) is almost 10-fold higher than wheat (*Triticum aestivum*) (41 mg/100 g), maize (*Zea mays*) (26 mg/100 g) and rice (*Oryza sativa*) (33 mg/100 g) and three times higher than milk (Kumar et al., 2016). So finger millet is an example of Ca rich crop in developing countries of tropical and subtropical regions. Finger millet grains also contain higher content of minerals such as phosphorus, iron and manganese compared to other cereals (Kumar et al., 2016). The seeds can be stored for more than 5 years without insect damage that makes it a valuable crop for famine hit areas (Latha et al., 2005). Crops such as rice and wheat provide food security, but finger millet provides nutritional security to the world (Devi et al., 2014; Ceasar et al., 2018).

Plants uptake Ca by root and translocate to the aerial parts of plants including fruits and seeds by symplastic and apoplastic pathways (Kumar et al., 2014; Mirza et al., 2014). In finger millet, Ca is present in aleurone layer followed by seed coat and embryo (Nath et al., 2013). The Ca transporters are actively involved in the uptake and transport of Ca in the cells, while Ca sensors are involved in the regulation of Ca transporters. Only a few Ca transporters have been identified before the releasing of draft genome sequence in finger millet. A few molecular marker-based studies have been conducted to identify the quantitative trait loci (QTL) for Ca traits in finger millet. In this review, we describe the nutritional importance and health benefits of finger millet. We have provided the detailed information on Ca sensor, accumulation and transport genes and identification of QTL related to Ca traits in finger millet.

NUTRITIONAL IMPORTANCE AND HEALTH BENEFITS OF FINGER MILLET

Finger millets serve as a major food in resource-poor countries of Asia and Africa by providing 75% of total calorie intake next to fine cereal grains (Singh et al., 2019) and gluten-free cereal products (Chandrasekara et al., 2012; Saleh et al., 2013). It is nutritionally superior to rice and wheat due to high amount of dietary fibers, iron, zinc, Ca, phosphorus, potassium, vitamin B, and essential amino acids (Parameswaran and Sadasivam, 1994; Ceasar and Ignacimuthu, 2011). Finger millet also possesses more lysine, threonine, and valine than

Abbreviations: Ca, Calcium; CaM, calmodulin; CaMK, CaM dependent protein kinase; CAX, Ca²⁺ cation exchanger/antiporter; CBL, Calciuneurin B-like protein; CDPK, CaM independent protein kinases; CIPK, Calciuneurin B-like protein (CBL)-interacting protein kinases; DAS, days after sowing; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; TPC1, two pore channel.



other millets (Ravindran, 1991; Sripriya et al., 1997). It has anti-nutrients including phytates, polyphenols, tannins, trypsin inhibitory factors and dietary fibers. Finger millet's dietary fiber and polyphenols offer various health benefits like antidiabetic, antioxidant, hypocholesterolaemic, antimicrobial, delayed nutrient absorption, increased fecal bulk, lowering of blood lipids effects (Devi et al., 2014). Over 50 phenolic compounds belonging to several classes such as phenolic acids and their derivatives, dehydrodiferulates and dehydrotriferulates, flavan-3-ol monomers and dimers, flavonols, flavones, and flavanonols were identified in finger millet (Saleh et al., 2013).

Finger millet's phenolic compounds like gallic, protocatechuic, p-hydroxy benzoic, p-coumaric, vanillic, syringic, ferulic, trans-cinnamic acids, and quercetin exhibit major antidiabetic and antioxidant properties and also inhibit cataract effectively (Saleh et al., 2013). Finger millet yields less glucose than the polished rice which is good for diabetic patients (Wang et al., 2018). Consumption of finger millet based diets resulted in significantly lower plasma glucose levels due to its higher fiber content and antinutritional factors than rice and wheat (Kumari and Sumathi, 2002). It also has beneficial effect on nerve growth factor production and wound healing properties at early diabetic condition (Rajasekaran et al., 2004). The grains and various fractions of finger millet have the potentials to prevent and treat diabetics (Saleh et al., 2013; Devi et al., 2014; Kam et al., 2016). Globally, most countries face high and increasing rates of cardiovascular diseases due to obesity, smoking, unhealthy diet, and lack of physical activity. This reduce plasma triglycerides in hyperlipidemic rats and prevent cardiovascular disease (Lee et al., 2010). The antioxidants and phenolics rich finger millet grains can also contribute to improve health, to protect against aging and metabolic syndrome (Bravo, 1998). Seed coat extracts of finger millet showed antimicrobial activity against *Bacillus cereus* and *Aspergillus flavus* (Radhajejalakshmi et al., 2003).

IDENTIFICATION OF QTL FOR CA TRAITS IN FINGER MILLET

Molecular markers are one of the most crucial diagnostic tools for plant breeding programmes. It is used to analyse genomes and enables the study of the genetic structure and traits of crop genomes. These markers are used to identify the specific genes responsible for better growth, development and resistance to abiotic and biotic stresses. The microsatellite markers have been used to identify the agronomically important traits such as grain yield, quality, disease resistance, drought resistance, nutritional quality, nutritional use-efficiency, etc (Maharajan et al., 2018). The availability of simple sequence repeats (SSR) markers in finger millet is limited compare to those for other cereal crops (Ceasar et al., 2018; Krishna et al., 2018). Only few QTL associated with Ca traits have been reported in finger millet. For example, 23 anchored SSR markers were used to identify nine QTL associated with Ca content in 113 genotypes of finger millet by association mapping (Kumar et al., 2015b). Recently, Yadav et al. (2020) identified two QTL (UGEP78 and UGEP60) for grain calcium content in 238 genotypes of finger millet through association mapping using 85 SSR markers. They also identified two genotypes with the highest grain calcium content [GPHCPB45 (452.8 mg) and (IE 2957 (447 mg))] among the 238 genotypes. This type of study is needed to detect high Ca content genotypes from more than 28,000 accessions of finger millet. The detection of genotypes with high Ca content may help to eradicate Ca deficiency around the world, particularly in Asian and African countries. Only association mapping populations were used for QTL studies so far in finger millet. There is a crucial need to develop the linkage maps for finger millet for the identification of QTL for the agronomically important traits including Ca. High-resolution research on the identification of QTL in finger millet for agronomically important trait is essential

for molecular breeding and finger millet improvement. The advanced backcross QTL (AB-QTL) methods are also helpful for a variety development (Kumari et al., 2020). Methods of AB-QTL analysis have been further used to identify and transfer the valuable QTL from un-adapted to cultivated germplasm in a single process (Wang and Chee, 2010; Bhanu et al., 2017). Back cross population (BC₂ and BC₃) are used for detection of QTL in this method. If worked effectively, AB-QTL analysis can prove to be a potential method to exploit unadapted and exotic germplasm for the quantitative trait improvement of a number of crop plants including finger millet. The released finger millet draft genome provides excellent opportunities to identify expressed sequence tag derived SSR markers for crop improvement (Hittalmani et al., 2017; Ceasar et al., 2018; Pandian and Ramesh, 2019).

GENOME-WIDE ASSOCIATION STUDIES FOR THE ENRICHMENT OF NUTRITIVE VALUE IN FINGER MILLET

The introduction of next-genome sequencing (NGS) technology helps for rapid and accurate detection of the genetic basis of phenotypic variation in crops. Due to its significant contribution to genome analysis, it can aid in understanding the genome organization of crops. NGS technology is useful to identify the genetic variability across the genomes of individuals and identify their genotype-phenotype associations in the huge germplasm of the crops. Therefore, genome sequencing plays a vital role in GWAS for crop improvement through a genome-assisted breeding (GAB) program. A very little effort was paid on GWAS in finger millet and only one report is available till date. Six nutritional traits such as iron, zinc, Ca, magnesium, potassium, and sodium were evaluated through GWAS using 190 finger millet genotypes and 418 single nucleotide polymorphism (SNP) linked with nutritional traits were identified (Swati et al., 2020). Out of 418 SNP, 18 SNP markers showed homology with candidate genes having putative functions in binding, remobilization/transport of metal ions in finger millet (Swati et al., 2020). This study provides the insight to genotypic variation of the phenotypes for the selection of the best breeding materials to improve grain nutritional content and nutrient use-efficiency in finger millet. Researchers need to pay more attention to GWAS of finger millet for improving their nutritive traits which may contribute to human health in the future. Furthermore, genotyping by sequencing (GBS) technology generated SNP markers are used to analyze the agro-morphological traits of 113 finger millet accessions and identify consistent SNP markers linked to grain yield and its component traits (Sharma et al., 2018). The identified SNP was associated with the candidate genes of rice and foxtail millet, which were responsible for flowering, maturity, and grain yield (Sharma et al., 2018). In another recent report, Tiwari et al. (2020) identified QTL and genes for seed protein content, days to maturity and grain yield in finger millet using 2977 SNPs by GBS technology. Among these, five SNPs were associated with seed protein content and grain yield and three for days to maturity. They have identified four candidate genes responsible for seed protein content using SNPs

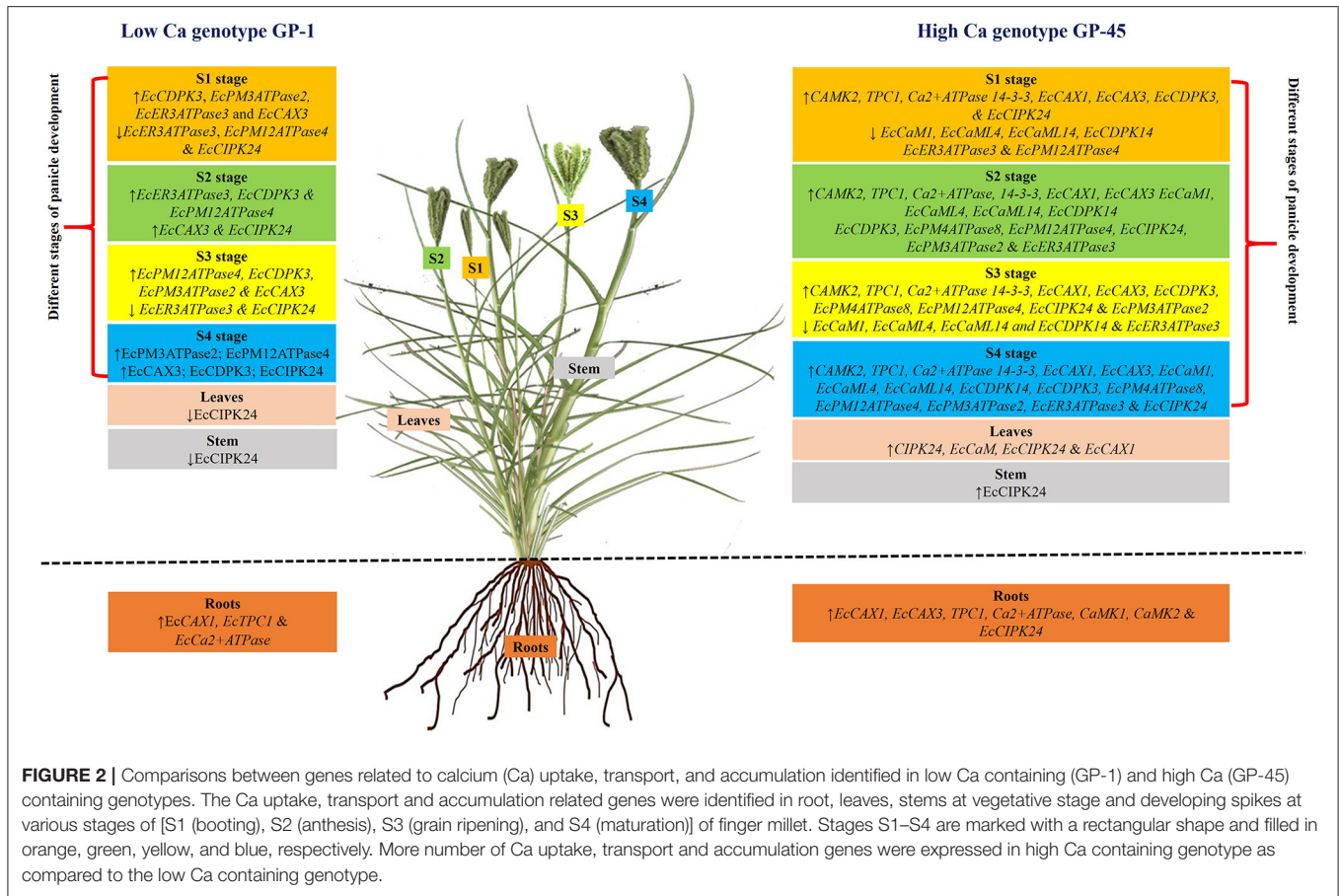
through *in-silico* analysis (Tiwari et al., 2020). Notably, *aspartyl protease* has been found to be the most promising candidate gene for seed protein content of finger millet. The plant breeders and molecular biologists need to focus more on GWAS in finger millet since it would be a key tool of GAB for finger millet improvement in the future.

TRANSPORTERS AND SENSORS FOR CA ACCUMULATION IN FINGER MILLET

The Ca is taken up by roots from the soil solution in a divalent cation (Ca²⁺) form (White and Broadley, 2003). The primary roles of the Ca are maintaining chemical balance in the soil, reducing soil salinity, activating the plant growth regulating enzymes and improving the water penetration and disease resistance in plants. Ca also improves the absorption of the other nutrients by roots and their translocation within the plant (Hepler, 2005). Ca transporters are classified into three types viz. Ca²⁺ channel, Ca²⁺ ATPase, and Ca²⁺ cation exchanger/antiporter (CAX) (Sze et al., 2000; Demidchik et al., 2018). These transporters export Ca²⁺ to the apoplast and into the cellular organelles specifically to the endoplasmic reticulum, golgi/endosome/pre-vacuolar compartments, and plastids/vacuoles (Karley and White, 2009). Plants absorb Ca by roots from the soil solution, which reaches the shoots through the xylem stream.

Two contrasting finger millet genotypes (GP-1; low Ca containing and GP-45; high Ca containing) were used to identify the expression pattern of *CAX1*, two pore channel (*TPC1*), *calmodulin* (*CaM*), *Ca²⁺ATPase*, and *CaM dependent protein kinase* (*CaMK1* and 2) genes in vegetative tissues and various stages of developing spike in finger millet (Mirza et al., 2014). This study laid the foundation for the identification of Ca transporter genes in finger millet and same genotypes were also utilized in the following studies. Same two genotypes were used to identify two *CaM independent protein kinases* (*CDPK3* and 13), four *Calcineurin B-like protein (CBL)-interacting protein kinases* (*CIPK2*, 4, 10, and 14), one *CaM*, one *TPC1*, two *CAXs* (*CAX1* and 3) and four *ATPases* (*CaATPase*, *CaATPase1*, 6 and 9) transporters in developing spikes of finger millet (Kumar et al., 2015a). *CIPK10*, *CDPK13*, *CIPK4*, *CaATPase*, *TPC1* and *CAX3* and *CaM*, *CDPK3*, *CIPK2*, *CaATPase6*, 9, *CAX1*, and 3 were expressed in developing spikes of GP-45 at S3 and S4 stages respectively (Figure 2). *CDPK3*, *CaM*, *CIPK14*, *CaATPase1*, *TPC1*, and *CAX1* were expressed in the developing spikes of GP-1 at S4 stage. Among these, expression level of *CIPK14* and *CaATPase1* were higher in GP-1 at S4 stage compared to other genes (Kumar et al., 2015a). This basic study revealed the spatial and temporal expression of these genes. Therefore, further study is required to characterize both the genes in GP-1 which may help to improve the low Ca containing genotype. The above results show that most of the Ca transport and accumulation genes were expressed in high Ca genotype compared to the low Ca genotype.

Likewise, 19 Ca transporter genes, which includes 11 *Ca²⁺ATPases*, seven Ca exchangers and one *Ca²⁺ channel* genes were identified in GP-1 and GP-45 through RNA-seq



(Singh et al., 2015). RNA-seq analysis revealed that nine (*EcVcax3*, *EcER3ATPase3*, *EcPM12ATPase4*, *EcPM8ATPase*, *EcPM5ATPase4*, *EcPM4ATPase8*, *EcPM3ATPaseB*, *EcPM3ATPase2*, and *EcPM2ATPase1*) and three (*EcCaX1b*, *EcCL1pore*, and *EcPM12ATPase4*) genes were highly expressed in GP-45 and GP-1 genotypes, respectively. The *EcPM4ATPase8* was highly expressed in developing spike of GP-45 at S2 and S3 stages under 20 mM and 5 mM Ca concentrations, respectively. Ca accumulation in seed is a polygenic trait and Ca²⁺ transporters (especially *EcCAX3*) might play very important role in seed Ca accumulation (Singh et al., 2015). This study reported first time an extensive list of Ca²⁺ sensing and transport related genes in finger millet. However, functions of these gene have not yet been validated by knock-out and knock-in studies. Another study shows that Ca sensor gene *CIPK* (*EcCIPK24*) was highly expressed in root, shoot, leaves and developing spike of GP-45 compared to GP-1 (Figure 2) (Chinchole et al., 2017). *CIPK* activates the *CAX* protein by interaction with *CBL* proteins. Docking analysis for *EcCIPK24* protein of the both genotypes with *CBL* proteins (*EcCBL4* and 10) were performed by Chinchole et al. (2017). The docking study proposed that *EcCBL4* has a strong binding affinity with *EcCIPK24* and might play a significant role in the accumulation of Ca in seeds (Chinchole et al., 2017). These predictions reveal the functions of the key Ca sensor gene in finger millet and further functional

characterization of this gene may help to understand the specific role in finger millet Ca accumulation.

In another study, *CAX1*, *CAX3*, *CaM*, *CBL4*, *CBL10*, and *CIPK24* genes were identified in roots, stem, leaves and developing spike of GP-1 and GP-45 (Figure 2) (Kokane et al., 2018). Most of the genes were highly expressed in roots (*CAX1* and *CAX3*), leaves (*CIPK24*), and stems (*CIPK24*, *CaM*, and *CAX1*) of GP-45 genotype compared to GP-1. Much of these works were carried out before the availability of draft genome of finger millet. So the full length gene sequences were not used in designing the primers and other molecular studies. Draft genomes of finger millet were released in 2017 by two groups (Hittalmani et al., 2017; Hatakeyama et al., 2018). Many reviewers have suggested that draft genomes of finger millet will help to improve the finger millet growth and yield through various molecular studies under both biotic and abiotic stress (Ceasar et al., 2018; Vetriventhan et al., 2020; Wambi et al., 2020). Hittalmani et al. (2017) identified 330 Ca transporter and accumulation related genes (28 *CaM ATPase*, 145 *CaMK1*, 125 *CaMK2*, 29 *CAX1*, and 3 *TPC1*) genes through transcriptome analysis. The identified genes will help in exploring more finger millet genotypes for Ca uptake, translocation and accumulation studies in future.

Apart from preliminary studies mentioned above, Ca transport and accumulation related genes have not yet been

studied extensively. Overexpression of Ca-signaling transporter genes is directly proportional to increased levels of Ca (Demidchik et al., 2018). For example, overexpression of *CAX1* transporter in *Arabidopsis thaliana* (Hirschi, 2001) and rice (Kim et al., 2005) enhanced the Ca levels in seeds of both the plants. Ca is acquired by root from the soil solution in free ionic form. Once Ca enters inside the root epidermal cells, it can move both apoplastically and symplastically to the cortex and then to the stele (Kumar et al., 2015c). Members of Ca sensors, exchangers and transporters are involved in Ca uptake, transport and accumulation at cellular levels in plants. Seed consists of maternal and filial tissues with no direct vascular connection between them (Wolswinkel et al., 1992). Ca is deposited in seed during its development which is mediated by specialized Ca transporters. It gets pumped toward the apoplast from seed coat, where it is absorbed by aleurone and then in endodermal cell (Sharma et al., 2017). Therefore, further studies to characterize the Ca sensors, exchangers and transporters may help to improve the finger millet growth and especially to accumulate Ca in seeds. When compared to research on Ca transporter family genes in finger millet to that of *A. thaliana* and rice, very little research has been carried out. Further study on Ca transporters' structure, function, affinity, and other molecular studies will throw more light on the complex function of Ca transporters in Ca uptake, transport, remobilization, and utilization in finger millet. Future high-resolution study on various aspects of Ca transporters in finger millet and the future development of new finger millet varieties with high Ca content will surely aid in enhancing nutritional security of the poor in the developing world.

CONCLUSION AND FUTURE PROSPECTS

Crop improvement has been one of the major priority areas of research in agriculture. Ca deficiency is a widespread problem across the globe, however, very little effort has been made to understand the mechanisms Ca accumulation in finger millet seeds. It may be due the reason that finger millet is being grown and consumed by less developed regions in the

world. Further study on the post-transcriptional and post-translational regulation of Ca transporters needs to be fine-tuned to exploit them in finger millet production to meet the ever increasing global population and food demand. Recently, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) gene-editing tool is projected to play a vital role in crop improvement. CRISPR/Cas9 tool could be applied to Ca transporter genes in future to dissect and improve the Ca accumulation in seeds of finger millet. Finger millet genotype with high Ca content will help to reduce Ca deficiency worldwide.

Bio-fortification is a food based approach to overcome the nutrient starvation by delivering nutrient dense crops at the door steps of poor populations. Bio-fortified crops, particularly finger millet contain high amount of proteins, essential amino acids, minerals, and vitamins which can help to reduce hidden hunger in the most vulnerable populations in the world. Finger millet is called as a super cereal because it is the richest source of Ca among all the cereals. Farmers who grow bio-fortified crops including finger millet can easily access the nutritious foods with minimal investments. Hence the incorporation of naturally Ca-rich cereals like finger millet into global bio-enrichment programs can be a good starting point to alleviate Ca malnutrition.

AUTHOR CONTRIBUTIONS

TM, TA, and SA conceptualized, wrote, edited, and updated the manuscript. SA and SI contributed critically in revising and improving the manuscript for publication. All authors contributed to the article and approved the submitted version.

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