Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 6(3) pp. 075-077, March, 2017 Issue.
Available online http://garj.org/garjas/home

Short Communication

Akt1 Molecular Identification in Bell Pepper

Pedro Alberto Rojas-Rojas[1], Sixto Velarde-Felix[2], Luis Alberto Lightbourn Rojas[1], and *Saúl Parra-Terraza[1].

[1]Plant nutrition, Facultad de Agronomía, Universidad Autónoma de Sinaloa, Culiacán, Sin., México.
[2]National Institute of Forest Agricultural Research And Livestock (INIFAP). And Road Culiacan-Eldorado Km. 17.5, Cp 80000. Culiacan Sinaloa. Mexico.

Accepted 14 March, 2017

Ion channels play a fundamental role in maintaining cellular homeostasis, which is one of the most important processes in plant production. Potassium channels are particularly interesting as they transport nutrients, used by cells in physiological processes. The purpose of this work was detecting the gene codes for the potassium ion channel AKT1 in roots of bell pepper seedlings, the PCR was standardized for AKT1 detection, and the oligo: AAGATCAGATGCACCTTGACTT was synthesized (best aligned at a temperature of 62 °C), then it was sequenced and analyzed presenting an identity of 94-97% at NCBI with the gene AKT1.

Keywords: Plant nutrition, Capsicum, PCR, gene, sequence.

INTRODUCTION

The specific assimilation of potassium (K^+) in the plant cell is due to membrane proteins called "ion channels" that provide cell homeostasis (Anschütz *et al*., 2014; Zörb *et al*., 2014). AKT1, KAT1 and KAT2, facilitate the entry of K^+ into the cell, they are sensitive to the outside concentration of K^+ (Meinke *et al*., 1998, Garriga *et al*., 2017). Potassium ion (K^+) is the most abundant nutritional element in plant parenchyma cells (Xu *et al*., 2002), this nutrient comprises more than 10% of the total dry matter of the plant due the root´s exploration. Nevertheless, potassium uptake kinetics were described more than 50 years ago (Martinez-Cordero *et al*., 2005).

K^+ is absorbed by the membrane's ion channels mainly in roots, they are activated by electrical action in response to the presence of external K^+ from cells, ranging from 10 µM to 10 mM (Maathuis and Sanders, 1994). The absorption is more common in ionic form by the root of inorganic sources mainly in monovalent elements, K^+ is an important cofactor of enzymes, carbohydrates and proteins of the metabolism (Mengutay *et al*., 2013), K^+ Participates in physiological functions, such as pH regulation and osmotic regulation of plants, this osmotic function is not exclusive to K^+ but it is the most important for maintaining water balance in the cells. Protein synthesis in the phloem in the form of malate that is decarboxylated in pyruvate and HCO_3 (which generates an increase in the pH of the rhizosphere and is exchanged for NO_3, the main component of the protein) has a fundamental role in photosynthesis due to its participation in the production of adenosine triphosphate (ATP) necessary for the conversion of CO_2 into glucose. It also participates in the fixation of atmospheric N_2 in legumes due to its capacity to recycle soil K^+ (Giacomini *et al*., 2003), transport and store assimilates and regulate the stomatal opening and closure by means of increased potassium in the guard cells, favoring the absorption of water from the adjacent cells to

*Corresponding Author's Email: saul.parra@uas.edu.mx

increase the turgidity of the cell causing the stomatal opening (Kant *et al*., 2005). An adequate level of K^+ also reduces the attack of fungi, bacteria, viruses and nematodes on plants mainly by synthesis of jasmonates and glucosinolates (Zörb *et al*., 2014), Besides that, accumulation of leaf sugars makes it more palatable for some insects, and bacteria could enter by the stomata under K^+ starvation (Melotto *et al*., 2006).

The absence of K^+ increases sugars in the leaves, which may replace osmotic molecules (Zörb *et al*., 2014). The uptake of K^+ is regulated by cotransporters at low concentrations and ion channels at high concentrations. The aim of this investigation is to standardize the method of detection of the AKT1 potassium ion channel gene by PCR for the identification of the gene coding for the AKT1 protein in bell pepper.

MATERIALS AND METHODS

The study was carried out at National Agricultural Research Institute (INIFAP) located in the municipality of Culiacán, Mexico, at 24º63 'north latitude and 107º44' west longitude, with 22 MASL. The outdoor temperatures averaged 20 ºC with maximum temperatures of 28 ºC and minimum temperatures of 16 ºC during the period from November 25, 2014 to January 17, 2015. The seeds used were *A. thaliana* var "Columbia" obtained from Colégio de Postgraduados Campus “Montecillo”, México City and bell pepper (*C. annuum* L.) var. "Cannon", booths were planted in polystyrene seedlings trays of 200 pots with individual volume of 30 cm^3 containing peat moss as substrate, germinated in a ventilated polycarbonate greenhouse with an area of 4 x 5 m, covered with black shade mesh (50%), the polystyrene trays were disinfected with 1% sodium hypochlorite for 15 minutes and the substrate used was sterilized in the autoclave for 2 hours, the management of the nutrient solution (SN) of seedling was balanced according to the “Steiner solution”, with reagent grade inorganic fertilizers and distilled water. Ten days after sowing (first true leaves) fertilization started, the concentration was increased every 10 days in 50%, 75% and 100%. The seedlings were watered daily at 08:00 and 14:00 h, sprinkling the foliage with SN (1 L capacity) to the solution runoff through the lower holes of the pots.

Forty-five days after sowing, the treatments were evaluated with fifty seedlings and three repetitions for each treatment, performing molecular biology analysis in each one as well. Before that, the seedlings from the polystyrene tray were extracted and leaflets and roots were separated and placed in a Taylor Wharton CryoScience Nitrogen tank with liquid nitrogen at -20 ºC to protect their genetic integrity during their transportation to the laboratory. Genomic DNA extraction was isolated in leaves and roots of pepper and*Arabidopsis* (Sanger *et al*., 1977). Once the DNA was obtained, a PCR amplification reaction of the AKT1 gene was performed, Primer obtain with Multialin Program, Primer3: primer tool, and Pepper Genome Platform, using Arabidopsis thaliana genomic (Li *et al*. 2006). A thermocycler was used For PCR (Nyx Technik Amplitronyx Series 6 A6 (ATC401) Thermal Cycler). After amplification of the PCR products, they were electrophoretically separated in 1% agarose gels (Brody and Scott, 2004).The purified PCR product was sent to the National Laboratory of Genomics for Biodiversity (LANGEBIO), The search for similarity between DNA sequences was performed using the BLAST program, which compared the nucleotide sequences under study with the National Center of Biotechnology Information (NCBI) databases (http: // www . Ncbi.nlm.nih.gov/BLAST/).

RESULTS AND DISCUSSION

Leaf and root genetic material analyzed by PCR shows the result of DNA quality and integrity for Arabidopsis thaliana (Meinke *et al*., 1998), and *Capsicum annuum*. The cleaning of DNA was different in root and leaf, due to the cleaning process (Sanger *et al*., 1977), the genetic material from leaves presented several macromolecules in a green color, as a result of that, only genetic material of the root was chosen, which is the organ of the seedling that responds to potassium due to its nutrient uptake (Maathuis and Sanders, 1994) Figure. 1. The results of DNA material quality and integrity showed higher definition in the roots (Brody and Scott, 2004), the AKT1 fragment was detected in roots of *Arabidopsis thaliana* as positive amplification results for AKT1 gene were also observed in bell pepper roots Figure. 1., Li *et al*. (2006) reported a sequence for identification of AKT gene in *A. thaliana*, that sequence generates an oligo which was synthesized and tested in temperature and concentration gradients, resulting in 26 good quality positive amplicons out of a total of 272 PCRs, with 26 positives, of which 17 correspond to oligo with the direct sequence: (AAG ATC AGA TGC TTG CAC ACTT) and reverse: (GCT TGA ACG GAT AGC TTT AGGA) (65% of hybridization), the highest positive incidence was observed with 35% of positive amplicons at 62°C. Showing the same number of incidences at the concentration of 100 ng -1, 50 ng -1 and 33 ng -1, whereas at 63 ºC it is observed that the amplification response varies as a function of the concentration, the amplification result was checked in the electrophoretic chamber, and amplified in 192 PCR reactions, which were subsequently purified with the extraction kit and collected until 50 µL of the PCR product was obtained at a concentration of about 100 ng / µL.

We obtained the direct sequence: (TTT CTT TTC TAC TCT TTA GTA GAT AAG GGT TTA CTT GTT TCG GGA GTA TCA AAC GAT CTA CAG TCG AGT CTC TTC CTA AAG CCA TCC GTT CAA GCG TTT CAC ATT TTC TTT

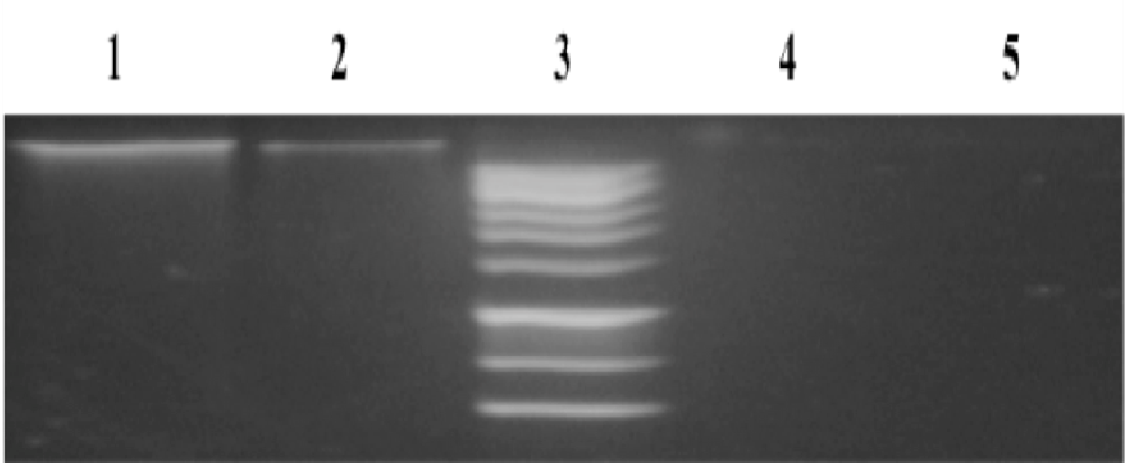

Figure 1. DNA Quality and Integrity. The 1st lane shows DNA pepper roots (1000 ng $^{-1}$), 2nd lane shows DNA pepper roots (100 ng $^{-1}$), 3rd lane shows molecular size marker HyperLader III, 4th lane shows DNA pepper leaf (1000 ng $^{-1}$) and 5th lane shows DNA pepper leaf (100 ng $^{-1}$).

TCT ACT CTT TAG TAG), which contributes to understanding molecular diversities of plants (Anschütz *et al*., 2014). The sequence obtained from pepper seedlings showed a similarity of 97% with the report (GenBank NM_001288418.1) in low affinity K^+ uptake, while Martinez-Cordero *et al*. (2005) reported AKT1 in high affinity K+ uptake which is essential under K^+ limiting conditions in *Arabidopsis thaliana*.

The identity of the sequences of the amplified fragments was analyzed with the Local Type Sequence Alignment Computer Program (BLAST) of the National Center for Biotechnology Information (NCBI). It showed a percentage of 99% identity with AKT1.

CONCLUSIONS

The procedure for the identification of the gene coding for the AKT1 protein present in pepper seedlings roots was standardized with the AAG ATC AGA TGC TTG CAC ACTT oligo at a temperature of 62 °C.

The sequence obtained was 42 nucleotides that were compared in the database where we matched them with 9 previous reports of AKT1.

REFERENCE

Anschütz U, Becker D, Shabala S (2014). Going beyond nutrition: Regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. Journal of Plant Physiology, 171(9), 670–687.

Brody JRy, Scott EK (2004). Sodium boric acid: A tris-free, cooler conductive medium for DNA electrophoresis. Biotechniques 36 (2):2-4.

Garriga M, Raddatz N, Véry A, Sentenac H, Rubio-meléndez ME, González W, Dreyer I (2017). Cloning and functional characterization of HKT1 and AKT1 genes of Fragaria spp .— Relationship to plant response to salt stress, 210, 9–17.

Giacomini SJ, Aita C, Vendruscolo ERO, Cubilla M, Nicoloso RS, Fries MR (2003). Matéria seca, relação C/N e acúmulo de nitrogênio, fósforo e potássio em misturas de plantas de cobertura de solo. Revista Brasileira de Ciência do Solo, 27(2), 325-334.

Kant S, Kant P, Kafkafi U (2005). Potassium uptake by higher plants: from field application to membrane transport. Acta Hungarica, 53(4). pp 43-459. Budapest.

Li L, Kim B, Cheong Y, Pandey GK, Luan S (2006). A Ca(2)+ signaling pathway regulates a K(+) channel for low-K response in Arabidopsis. Proc. Natl Acad. Sci. USA, 103, 12625–12630.

Maathuis F, Sanders D (1994). Mechanism of high-affinity potassium uptake in roots of Arabidopsis thaliana. Vol.91, pp. 9272-9276, Department of Biology, University of York, United Kingdom.

Martínez-Cordero MA, Martínez V, Rubio F (2005). High-affinity K+ uptake in pepper plants. Journal of Experimental Botany, 56(416), 1553-1562.

Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M (1998). Arabidopsis thaliana: a model plant for genome analysis. Science, 282(5389), 662-682.

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006). Plant stomata function in innate immunity against bacterial invasion. Cell ;126:969–80.

Mengutay M, Ceylan Y, Kutman UB, Cakmak I (2013). Adequate magnesium nutrition mitigates adverse effects of heat stress on maize and wheat. Plant and Soil. vol. 368. no. 1, pp. 57-72.

Sanger F, Nicklen S, Chase AR (1977). DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Sciences. 74(12):5463-5468.

Xu G, Wolf S, Kafkafi U (2002). Ammonium on potassium interaction in sweet pepper. Journal of Plant Nutrition, 25(4), 719.

Zörb C, Senbayram M, Peiter E (2014). Potassium in agriculture – Status and perspectives. Journal of Plant Physiology, 171(9), 656–669. •