

EgKT2-1, an inwardly rectifying Shaker-type potassium channel in oil palm is involved in oil production

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Abstract

The oil extracted from the mesocarp of oil palm (*Elaeis guineensis*, Jacq.) drupes is the world's leading source of vegetable fat. Oil palm cultivation requires large quantities of mineral nutrients supplied through fertilization, which represents a major production cost. Among essential nutrients, potassium (K^+) plays a critical role in plant growth and fruit development. However, the mechanisms of K^+ transport and its specific contribution to yield remain poorly understood. This study focuses on the identification and characterization of a *Shaker-like* potassium channel, EgKT2-1, which belongs to the AKT2-like subfamily. Gene expression analyses, using quantitative PCR, revealed that *EgKT2-1* is strongly expressed in leaves, meristem, and young fruits correlated with increased K^+ accumulation during the early stages of fruit development. *In situ* hybridization further localized *EgKT2-1* transcripts in the cortex and phloem of roots, the palisade parenchyma of leaves, and the mesocarp cells of young fruits. These results suggest that EgKT2-1 participates in K^+ loading and transport between source tissues and sink organs. Interestingly, even though EgKT2-1 belongs par homology to the weakly rectifying AKT2-like subfamily, electrophysiological characterization revealed an inwardly rectifying ion channel activity pointing to a role in massive K^+ uptake. Taking together, the original results showing that EgKT2-1 is an inward rectifier channel and that higher expression of *EgKT2-1* was observed in a cross with higher oil yield might point to an increase in the availability of K^+ in mesocarp cells for oil biosynthesis and, consequently, a direct correlation between the K^+ channel activity and oil production.

Key message

EgKT2-1, an inwardly rectifying *Shaker*-type potassium (K^+) channel in oil palm, is expressed in leaves, shoot meristem, and young fruits, and boosts K^+ availability in mesocarp cells for oil formation.

INTRODUCTION

The oil palm (*Elaeis guineensis*, Jacq.) is a monocotyledonous plant whose fruits are named drupes growing in bunches. The drupe is composed of a thick fleshy mesocarp, which is exceptionally rich in oil, surrounded by the hardened endocarp (shell), that encloses the kernel and a tiny embryo. Palm oil, which is extracted from the mesocarp is the world's leading source of vegetable fat (36%) and the crude oil is naturally rich in vitamin E and provitamin A (USDA ERS, 2022). Despite accounting for only 8.6% of the total area devoted to oilseed crops worldwide, oil palm exhibit an unmatched productivity (3,5 – 4,5 t of oil ha^{-1} year $^{-1}$), surpassing other oilseeds such as soya (0,5 – 0,6 t of oil ha^{-1} year $^{-1}$), sunflower (0,6 – 1,0 t of oil ha^{-1} year $^{-1}$) and rapeseed (0,8 – 1,0 t of oil ha^{-1} year $^{-1}$) (FAOSTAT, 2025; Murphy, 2025). Oil palm fruits ripen around 160 days after anthesis (DAA). During the early fruit development stages, *i.e.* between 30 and 60 DAA, cell division and expansion drive increases in fruit size and mass, followed by a latency phase (60–100 DAA) characterized by reduced hormonal activity (auxin, gibberellins, cytokinins). A decrease in auxin, gibberellic acid, and cytokinin hormones occurs before maturation. From 120 DAA onward, ripening begins with a strong simultaneous increase in ethylene and ABA and production of fatty

acids. Carotenes and oil are produced during maturation from 140 DAA (Tranbarger et al. 2011). To reach high yields, oil palm requires large quantities of fertilizer (Ollivier et al. 2017).

Among the provided inputs, potassium (K^+) is an essential macronutrient for oil palm, influencing vegetative growth, bunch and drupe production and overall yield (Corley and Tinker 2015; Husri et al. 2020). As the most abundant cytosolic cation, K^+ participate in highly integrated functions such as the control of stomatal opening and gas exchange, chlorophyll assimilation, cell turgidity, raw sap rise or phloem sap circulation, cell elongation, and plant growth. It plays also a key role in the plant's adaptation to variations in water availability in the environment. In oil palm plantations, in order to increase oil production, the average K^+ quantity supplied is $0.52t$ of K^+ fertilizer $ha^{-1} year^{-1}$, which can account for up to 50% of the total production costs (Husri et al. 2020). Optimizing K^+ use efficiency is thus critical to reducing fertilizer dependency and improving sustainability.

Some molecular knowledge of the development and maturation of the oil palm fruit has been reported in the last decade, although still relatively fragmentary, namely (1) the *de novo* synthesis of fatty acids and their assembly in the pulp in *E. guineensis* (Montoya et al. 2013), (2) the transcriptional basis of oil and carotenoid biosynthesis during mesocarp development and maturation in palm fruits (Tranbarger et al. 2011), and (3) a comparison of fatty acid and sugar synthesis in oil palm and date palms (Bourgis et al. 2011). It is well established that K^+ content in the fruit mesocarp increases progressively during the early stages of fruit formation up to 105 DAA then decreases during later development stages when lipid biosynthesis occurs (Desassis, 1961). However, but few data are available concerning K^+ transport and its involvement in fruit formation and yield. Generally, in plants, several families of transporters and ion channels transport K^+ , among which *Shaker*-like K^+ channels dominate the K^+ conductance at the plasma membrane. *Shaker*-type K^+ channels are voltage-dependent and are responsible for massive fluxes over long distances. These channels are involved in various biological processes such as the uptake of K^+ from the soil, its secretion into xylem sap and the redistribution of this ion by phloem sap (Dreyer and Uozumi 2011). Voltage-dependent *Shaker*-type channels share a common structure composed of six transmembrane domains (S1-S6) featuring a selective pore region with the characteristic "GYGD" motive between tS5 and S6 and a voltage sensor in S4. The transmembrane domains are followed by a large C-terminal region harboring a putative cyclic nucleotide-binding domain (CNBD) and a K_{HA} domain rich in acidic and hydrophobic amino acids involved in channel clustering (Ehrhardt et al. 1997; Pilot et al. 2003). Another motive within the C-terminus, not always present in all channels, is the ankyrin domain responsible for protein-protein interaction. *Shaker*-type channels are functional at the plasma membrane, when four monomers combine to form a central pore, either by homo-tetramerization, if the four subunits are identical, or by hetero-tetramerization by combining different subunits, which increases the functional diversity of the channel properties (Sharma et al. 2013; Véry et al. 2014). Plant *Shaker*-like channels are divided into five groups (I-V) according to their structural homologies and functional properties (Pilot et al. 2003; Gambale and Uozumi 2006). Specifically, these groups are distinguished by their current rectification, allowing directed K^+ fluxes mediated by K_{in} , K_{out} or K_{weak} channels. Groups I

and II are inwardly rectifying channels (K_{in}) that allow K^+ uptake into the cytosol when the membrane is hyperpolarized, differing by the presence or absence of an ankyrin domain. Group III forms weakly rectifying channels (K_{weak}) that can mediate both K^+ influx and efflux depending on the membrane potential and concentration gradient and operate through two gating modes. Group IV consists of silent subunits that are not functional alone but form hetero-tetramers with K_{in} or K_{weak} channels to regulate their activity. Finally, group V includes outwardly rectifying channels (K_{out}) activated by membrane depolarization, which drive K^+ efflux from the cytosol to the apoplast. Regarding transporters, in oil palm, three K^+ transporters belonging to the K^+ Transporter (KT), K^+ Uptake Permease (KUP) and High Affinity K^+ transporter (HAK) family have been identified and characterized (Husri and Ong-Abdullah 2018; Husri 2019), providing initial insights into K^+ uptake mechanisms. In fact, the KT/KUP/HAK transporter family is known to be important for plants when subjected to low K^+ concentrations (Nieves-Cordones et al. 2010). In oil palm, unlike the expression of *KUP8*, the expression of *KUP3* and *KUP11* does not appear to be sensitive to external K^+ in roots and leaves (Husri and Ong-Abdullah 2018). Despite evidence that K^+ content in the mesocarp peaks during early fruit development (up to 105 DAA) before declining when lipid biosynthesis starts (Desassis, 1961), the molecular mechanisms underlying K^+ transport, accumulation, and redistribution particularly via *Shaker*-type channels remain poorly understood. A complete dissection of these processes, both at the whole-plant level and during fruit development, is essential to improve K^+ use efficiency to enhance oil palm crop performance. To address these questions, we analyzed the *Shaker*-like K^+ channel family in oil palm and provide a first functional description of EgKT2-1, a member of the AKT2-like subfamily (Group III), which is highly expressed in fruits, whereas EgKT2-2 does not show any detectable expression in fruits, as indicated by the absence of transcripts in real-time PCR experiments (S.E. and T.C., unpublished data). We report the expression profile of *EgKT2-1*, its cellular localization analyzed by *in situ* hybridization and its functional characterization in *Xenopus laevis* oocytes. Comparative analyses between two contrasted crosses concerning the production of Fresh Fruit Bunches (FFB) and crude palm oil (CPO) (C2 and C3) suggest that *EgKT2-1* contributes to oil production, likely by enhancing K^+ availability in mesocarp cells during early fruit development. Altogether, this study offers critical insights for improving K^+ use efficiency ultimately aiming at reduction of fertilizer use and promote an eco-responsible management of oil palm groves.

MATERIAL AND METHODS

Plant materials

The experimental field was located at the Ologbo Estate of Presco Plc (Edo State, Nigeria, N 6.03652° E 5.55609° at an elevation of 20 m a.s.l). The trees were planted in 2011. The material from adults trees (flowers, fruits, leaves and rachis from leaves) came from two crosses (C2 and C3) of the same origin (Deli x La Mé). C2 from DA 115 D AF x (LM 5 T x LM 10 T) and C3 from DA 115 D AF x LM 5 T AF; AF refers to self-pollinated trees. These crosses were selected for this study because, although they belong to the same origin, they exhibit different yields with the same quantity of K^+ fertilizer in Nigeria (Dassou

et al. 2022a, b). The C3 progeny was recently reported to yield more oil and more bunches than the C2 progeny (Dassou et al. 2022a). Fruits were collected at three stages of development, young fruits at 30–60 DAA, mid maturity (around 120 DAA) and maturity (around 160 DAA). Each organ was collected from a total of 27 palms. Mesocarp, leaves and rachis tissues were placed in RNA stabilization reagent (RNAlater™, Qiagen) and stored at -20°C prior to RNA extraction. Approximately 100 mg of material were ground in liquid nitrogen and used for RNA extraction. The plants of Deli x La Mé origin were grown in the greenhouse for five months. The fresh tissues (young leaves, rachis, roots and meristem) were frozen in liquid nitrogen and stored at -80°C until RNA extraction.

Isolation of cDNA encoding EgKT2-1 protein

The reference genome sequence of oil palm (Jin et al. 2016) enabled *in silico* analysis for cloning of the selected *EgKT2-1* gene (LOC105036922). Specific primers (EgKT2-1-full-F 5'-ATGATGAATTGGTCCTTGAGCAAG-3 and EgKT2-1-full-R 5'-TCAAAGAAATTCTTCATTCTCAACTATGAAC-3) were designed based on the predicted sequences, and the corresponding cDNAs were isolated from leaf RNA.

RNA extraction and real time PCR analyses

The protocol proposed by Argout et al. (2008) was used to extract RNA from fruits. For the other tissues, total RNA was extracted using the Plant RNeasy extraction kit (Qiagen, Germany). After DNase I treatment (RQ1 RNase-free DNase, Promega, (Promega, Madison, WI, États-Unis; Cat. No. M610A), total RNA was eluted in 50 µl and quantified in an Agilent TapeStation 4200 System (Agilent Technologies, Santa Clara, CA, USA, <https://www.agilent.com>). Total RNA (500 ng) was used as the template to synthesize first strand cDNA with SuperScript III reverse transcriptase (Invitrogen™, Carlsbad, CA, USA; Invitrogen Cat. No. 18080-093), according to the manufacturer's instructions. Oligonucleotides were designed using Primer3Plus (<https://primer3plus.com>). All primers were designed in the 3' region of each gene. The tubulin alpha-3 chain (LOC105054774) gene (TUA5_F 5'-TGGAGAAGGGATGGAAGAAG-3' and TUA5_R 5'-GGGAGAGGGAGAGAGAAACC-3') was validated and used as housekeeping gene. The oligonucleotides used for *EgKT2-1* were KT2_F: 5'-ATACTCCCTTATGGCTCGCC-3' and KT2_R: 5'-CGAGAAGATTGCCACCGATG-3'. PCR reactions were performed on a LightCycler 480 II System (Roche Diagnostics, Mannheim, Germany) using SYBR Green I Master Mix ((Roche Diagnostics, Mannheim, Germany; Cat. No. 04707516001) and gene-specific primers in 384-well plates according to the manufacturer's specifications. Reactions were performed in duplicate with the automated system (Biomek FXp) and at least three palms were used as biological replicates. The relative expression values were calculated using the $2^{-\Delta Ct}$ method (Livak and Schmittgen 2001). Gene expression was compared with the tubulin gene (TUA) used as housekeeping gene and ΔCt was calculated as $\Delta Ct = Ct_{\text{Gen target}} - Ct_{\text{housekeeping}}$. The trend in the expression levels of each gene was plotted using the median value of the $2^{-\Delta Ct}$ data. Statistical differences between samples were evaluated using a Kruskal-Wallis non-parametric test, with a p-value < 0.05 considered significant.

Localization of EgKT2-1 expression by mRNA in situ hybridization

EgKT2-1-specific primers EgKT2-1_F and EgKT2-1R used for RT-qPCR were used to synthesize RNA probes according to the protocol described by Alemanno et al. (2008). Sense probes (used as controls) and antisense probes were labelled with UTP-digoxigenin during transcription (T7 MAXIScript Kit, Ambion, Austin, TX, USA). Fruits, roots and leaves were fixed overnight in 4% paraformaldehyde under RNase free conditions, dehydrated through an ethanol series, and transferred to butanol for two weeks prior to paraffin embedding (Leuty 1969; Ferreira et al. 2017). Samples were then sectioned into 10 µm-thick cross sections using a microtome (Leica RM2125), and mounted onto silanized slides (Dako, Trappes, France). Hybridization was performed overnight at 45°C. Slides were incubated with anti-DIG antibody combined with alkaline phosphatase (Roche Diagnostics, Mannheim, Germany; <http://www.roche.com>) diluted 1:500 for 1 h at 37°C. Vector blue KITIII (Vector Laboratories, <http://www.vectorlabs.com>) was used to detect hybridization signals. Signals were observed with a bright field microscope (Nikon Eclipse NI-E) equipped a 4x, 10x and 20x objectives. Images were captured using a DS-Ri2 camera (Nikon Instruments Inc., Tokyo, Japan), and scale bars were added using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Functional characterization of EgKT2-1

For functional characterization in *X. laevis* oocytes, *in vitro* transcriptions were performed using the mMESSAGE mMACHINE T7 Ultra Transcription Kit (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No. AM1345) following the manufacturer's instructions to obtain *EgKT2-1* cRNA. The *EgKT2-1* cRNA (6 ng in 12 nl) was microinjected into *Xenopus laevis* oocytes using a microinjector (Nanoliter 2010, WPI, <https://www.wpiinc.com>). Control oocytes received an equivalent volume of water (12 nl). Injected oocytes were stored at 20°C in ND96 solution (mM: NaCl 96, KCl 2, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, Na-pyruvate 2.5; gentamycine 50 µg ml⁻¹, pH 7.4). Oocyte's membrane currents were recorded 2–4 days postinjection, using a two-microelectrode voltage-clamp amplifier (GeneClamp 500B; Axon Instruments, Union City, CA, USA; <http://www.moleculardevices.com>), and a Digidata 1322A interface (Axon Instruments), with microelectrodes filled with 3 M KCl. Recordings were visualized and stored with pClampex 10 software (Molecular Devices, Sunnyvale, CA, USA).

During recordings, oocytes were maintained in perfusion solutions containing 100 (K100), 50 (K50), or 10 mM (K10) K⁺ gluconate, supplemented respectively with 0, 50 or 90 mM Na⁺ gluconate in: (mM) 1 CaCl₂, 2 MgCl₂, and 10 HEPES (pH 6.5 or 7.5) or 10 MES (pH 5.5). Channel selectivity was tested using (mM) KCl 100, NaCl 100, LiCl 100 and RbCl 100 solutions. Inhibition by Cs⁺ or Ba²⁺ was analyzed by adding 10 mM CsCl or BaCl₂ to the K100 solution at pH 6.5. A voltage-clamp protocol was applied with 1.4 s voltage pulses from +70 mV to -170 mV, in -15 mV steps, followed by a 250 ms voltage step to -40 mV at a holding potential of 0 mV. Current-voltage (I/V) curves were obtained by plotting steady-state currents measured at the end of voltage pulses against the corresponding membrane potentials. Data were analysed using Clampfit 10 software (Axon Instruments).

RESULTS

Phylogenetic relationships of EgKT2-1

Using the EguV3 reference genome (Jin et al. 2016), we identified eight sequences corresponding to genes encoding *Shaker*-type K^+ channels in oil palm and constructed a phylogenetic tree to classify them. Phylogenetic analyses confirmed that the *Shaker*-like K^+ channel family in oil palm includes eight members, consistent with the number described in date and coconut palms (Yang et al. 2021) while *Arabidopsis thaliana* (hereafter *Arabidopsis*) harbors 9 (Sharma et al. 2013) and rice 10 channels (Véry et al. 2014) (Fig. 1A). These oil palm channels were named as follows: four members; EgKT1-1, EgKT1-2, EgKAT1 and EgKAT2, belong to group I and II of the inwardly rectifying (K_{in}) channel subfamily, two subunits, EgKOR-1 and EgKOR-2, belong to group V of the outwardly rectifying (K_{out}) channel subfamily, and the remaining two members, EgKT2-1 and EgKT2-2, belong to group III of the weakly rectifying (K_{weak}) channel subfamily. Interestingly, oil palm like other palm species, completely lack the silent gene group (AtKC1-like) found in other model species. A notable feature in palms is the presence of two AKT2-like subfamily members (EgKT2-1 and EgKT2-2), whereas only a single member (AtKT2) has been described previously as weakly rectifying channel in the model species *Arabidopsis* (AtKT2) (black box in Fig. 1).

A phylogenetic tree was constructed using NGPhylogeny.fr software (Lemoine et al. 2019) (mode, selecting a combination of MAFFT, TrimAl and PhyML in a Galaxy Workflow) and the InTreeGreat web interface (Dufayard et al. 2021) using the complete set of *Shaker* channel polypeptides of *Arabidopsis*, rice and oil palm. The tree was manually pre-rooted with forester.jar application (<https://www.phylosoft.org/archaeopteryx>). *Shaker* channels are divided into five groups: Group I, the AKT1 subfamily, comprises inwardly rectifying channels with ankyrin domains. Group II, the KAT1 subfamily, harbors inwardly rectifying channels without the ankyrin domain. Group III, the AKT2 subfamily, is defined by channels with weakly rectifying K^+ channels. Group IV, the AtKC1 subfamily, includes subunits that modulate other inwardly rectifying channels. Group V, the SKOR/GORK subfamily, gathers outwardly rectifying K^+ channels. The *Arabidopsis* *Shaker*-like K^+ channel family has nine members, the rice family ten members. In oil palm, the *Shaker* K^+ channel family is composed of eight members. Note the presence of two oil palm members, EgKT2-1 and EgKT2-2, within group III, in the so-called AKT2-like subfamily (black box), whereas only one member was found in *Arabidopsis* and in rice, respectively. I Note the absence of group IV in oil palm.

NCBI RefSeq gene symbols: EgKT1-1 (LOC105047848), EgKT1-2 (LOC105047770), EgKAT1 (LOC105043816), EgKAT2 (LOC105040639), EgKT2-1 (LOC4325560), EgKT2-2 (LOC105051829), EgKOR1 (LOC105032653), EgKOR2 (LOC105051554); AKT1 (AT2G26650), SPIK (AT2G25600), AKT5 (AT4G32500), KAT1 (AT5G46240), KAT2 (AT4G18290), AKT2 (AT4G22200), AtKC1 (AT4G32650), GORK (AT5G37500), SKOR (AT3G02850); Os_AKT1 (LOC4326245), Os_AKT3 (LOC9271501), Os_KAT1

(LOC4328864), Os_KAT2 (LOC4325560), Os_KAT3 (LOC4325143), Os_AKT2 (LOC4338867), Os_KAT4 (LOC107276707), Os_KAT6 (LOC107276718), Os_KOR1 (LOC4340653), Os_KOR2 (LOC4335955).

In this study, we focalized on the characterization of one of the two AKT2-like channels in oil palm, named EgKT2-1, due to its high expression during the early stages of fruit development (cf. gene expression results). A 2511 bp full-length cDNA *EgKT2-1* (LOC105036922) was amplified using specific primers. The deduced EgKT2-1 polypeptide contains 836 amino acids and shares 73.86% and 68.71% identity with AtKT2 and OsKT2, respectively. EgKT2-1 has the typical characteristics of a *Shaker* channel: six transmembrane segments with the K⁺ selective hallmark sequence 'TXXTXGYGD', followed by a large C-terminal domain containing a cyclic nucleotide-binding domain (CNBD), an ankyrin domain and a K_{HA} domain. The second EgKT2 channel (EgKT2-2) encodes a deduced polypeptide of 833 amino acids and shares 63.19% identity with OsKT2 and only 56.9% with AtKT2. It also displays the typical characteristics of Shaker channels. The two EgKT2 channels share 71.2% sequence identity.

Differential EgKT2-1 expression across roots, leaves, and fruits underlies oil yield variation in oil palm crosses C2 and C3

Analyses of gene expression by RT-qPCR showed that *EgKT2-1* is ubiquitously expressed in all tested tissues. Notably, *EgKT2-1* was strongly expressed in leaves and meristems of young greenhouse plants. In adult palms, the highest expression levels were detected in leaves and young fruits (30–60 DAA). In contrast, *EgKT2-1* showed low expression levels in flowers, roots, rachis, mid-ripening fruits (around 120 DAA), and mature fruits (160 DAA) (Fig. 2A).

Figure 2A: **Expression of the EgKT2-1 channel in oil palm.** Relative abundance of *EgKT2-1* transcript levels in oil palm tissues. The expression levels of the *EgKT2-1* gene were investigated by RT-qPCR on total RNA extracted from leaves, rachis, meristem, and roots from young plants as well as from leaves, rachis, flowers and fruits in three developmental stages from adult palms. Analyses of gene expression levels showed that *EgKT2-1* was strongly expressed in leaves, meristem, and young fruits (30–60 DAA) coinciding with the reported increase in K⁺ in the early steps of fruit development. In contrast, *EgKT2-1* was weakly expressed in flowers, roots, rachis, mid-mature fruits (around 120 DAA) and mature fruits (160 DAA).

Moreover, we analyzed *EgKT2-1* expression levels in the fruits from two crosses C2 and C3. Both crosses are hybrids derived from the same parental populations (maternal Deli population x paternal La Mé population) and were cultivated in Nigeria under identical K⁺ fertilizer conditions. However, the C3 progeny produced significantly more oil and bunches than the C2 progeny under the same growing conditions (Dassou et al. 2022a, b). Remarkably, the expression level of *EgKT2-1* in fruits from the C3 cross was almost fourfold higher than that observed in fruits from the C2 cross (Fig. 2B), suggesting enhanced K⁺ uptake. This higher expression of *EgKT2-1* may imply a greater K⁺ influx into the mesocarp cells.

To further characterize the spatial expression pattern of *EgKT2-1*, we performed tissue localization studies using *in situ* hybridization (Fig. 3). No signal was detected with the sense probe used as a negative control in any tissue. In roots, transcripts were localized in the root cortex and phloem of young seedlings (Fig. 3A), as observed in both cross and longitudinal sections. Notably, strong labeling was also detected in the root pericycle, particularly in pericycle cells located adjacent to the xylem poles (Fig. 3A).

In leaves, hybridization signals were observed in palisade parenchyma cells (mesophyll) (Fig. 3B) whereas no labeling was detected in vascular bundles or stomata. In fruits, *in situ* hybridization was performed at three key developmental stages: young fruits (60 DAA), mid-ripening fruits (120 DAA), and mature fruits (160 DAA).

EgKT2-1 transcripts were exclusively localized in mesocarp cells of young fruits (60 DAA) (Fig. 3C) coinciding with K^+ accumulation during the early stages of fruit development. No signal was observed in mid-ripening and mature fruits (data not shown).

EgKT2-1 mediates inwardly rectifying K^+ currents in *Xenopus laevis* oocytes

The oil palm *Shaker*-like K^+ channel *EgKT2-1* was heterologously expressed in *X. laevis* oocytes and membrane currents were measured and compared to those of control oocytes (Fig. 4).

While water-injected control oocytes did not display any detectable currents, *EgKT2-1*-injected oocytes displayed voltage- and time-dependent inwardly rectifying currents upon membrane hyperpolarization in an external K^+ (100 mM) (Fig. 4A). These time-dependent activating currents showed sigmoidal activation kinetics, a hallmark of plant⁺ inwardly rectifying K^+ channels. Varying external K^+ concentrations caused a change in current amplitude with increased external K^+ resulting in higher current amplitudes (Fig. 4B) confirming a K^+ -dependent conductance. Notably, the activation potential remained at around -50 mV. Remarkably, large amplitudes of inward currents (up to -25 μ A) were observed, but outwardly directed (positive) currents remained negligible even at low external K^+ , indicating that residual currents likely correspond to endogenous or leak-like currents. Based on these findings that clearly revealed inwardly rectifying K^+ currents, the oil palm channel *EgKT2-1* belongs functionally to the subfamily of inward rectifiers and not to the weak rectifiers of the AKT2-type, as initially expected based on the phylogenetic relationship, which reflected sequence similarities. Further analyses of the selectivity of *EgKT2-1* were performed to test the permeability of other monovalent cations. Ion currents were compared in the same oocytes expressing the oil palm channel in external solutions of 100 mM K^+ , sodium (Na^+), lithium (Li^+) and rubidium (Rb^+) (all as XCl; Fig. 5).

Current traces in response to voltage-pulses (Fig. 5A) demonstrated reduced amplitudes for all the other cations tested including Rb^+ (K^+ channels in plants are partially permeable to rubidium), compared to the K^+ control. The mean currents plotted in current-voltage (IV) curves (Fig. 5B) showed reduced current intensities for Rb^+ compared to that of K^+ . In parallel, (almost) no current was recorded for Na^+ or Li^+ ,

suggesting that the remaining little currents were due to endogenous or leak-like currents. These results showed that EgKT2-1 is rather selective for K^+ ($K^+ > Rb^+ >> Li^+, Na^+$). It is also important to note that the activity of EgKT2-1 was not significantly sensitive to variations in external pH in the range of 5.5 to 7.5 in 100 mM external K^+ (Fig. S1 in supplementary data). The pharmacological properties of the channel were then analyzed by external perfusion using the K^+ channel blockers, Cs^+ and Ba^{2+} (Fig. 6A). The results showed that EgKT2-1 currents were largely inhibited at all current-activating membrane potentials. At -170 mV, the mean currents were reduced by 75% (10 mM CsCl, $n = 8$; Fig. 6B) and by 80% (10 mM $BaCl_2$, $n = 8$; Fig. 6C). It should be noted that the inhibitory effect of these blockers was reversible upon return to a 100 mM K^+ .

DISCUSSION

EgKT2-1 behaves as an inward K^+ channel

EgKT2-1 was initially phylogenetically classified in the group of AKT2-type channels (group III or weakly rectifying channel) like the other orthologues in the family based on sequence similarity (Fig. 1). The subfamily AKT2 group is characterized by unique electrophysiological properties showing only a weak rectification behavior. Within the oil palm genome, we identified two members of the AKT2 subfamily unlike the model species *Arabidopsis* or rice. Interestingly, the functional characterization of one of these members, namely of the EgKT2-1 channel, in *X. laevis* oocytes demonstrated clearly that it behaved differently from a standard weakly rectifying AKT2 channel, but mediated large inwardly rectifying K^+ currents (Fig. 4A), enabling massive cellular K^+ uptake. Similarly, Huang et al. (2021) reported recently the characterization of a *Shaker* channel in rice named OsAKT2 belonging to the AKT2 subfamily. This channel differs from the behavior of canonical AKT2 channels, so it acted mainly as an inward rectifier with strong voltage-dependency. These authors identified a single lysine residue at position 191 (K191) in the S4 domain, instead of an arginine (R) in *Arabidopsis*, as being responsible for controlling the behavior of the inward rectifier. Thus, they performed the K191R mutation in OsAKT2 that restored the typical bidirectional weak rectification. Reciprocally, the R191K mutation of the *Arabidopsis* AtAKT2 transformed this channel into an inward rectifier by losing its leak-like behavior, demonstrating that K191 is responsible for this switch between inward and weak rectifiers. Concerning the oil palm channel EgKT2-1, amino acid sequence alignments between AtAKT2, OsAKT2 and EgKT2-1 revealed a single K195 residue in EgKT2-1 similar to the K191 residue in OsAKT2 versus two continuous RR residues (R194 and R195) in *Arabidopsis* (Fig. S2 in supplementary data). As for OsAKT2, the presence of a single K195 residue in the S4 domain in EgKT2-1 similar to K191 argues for the control of the inward rectification. In addition, we examined the alignment of the barley HvAKT2 with AtAKT2, OsAKT2, and EgKT2-1 (Fig. S2 in supplementary data). The channel HvAKT2, like OsAKT2 and EgKT2-1, exhibits predominant inward currents (Boscari et al. 2009). As expected, like OsAKT2 and EgKT2-1, it has a unique K183 instead of two continuous RR residues like *Arabidopsis*, which once again confirms that this residue is responsible for the rectification behavior. Taken together, the initially concluded AKT2-like weak rectification, found by chance in the first model *Arabidopsis*, is not a general rule but dependent on

a specific amino acid in S4. Physiologically, strict inward rectification might be an advantage for K⁺ transport regulation in the studied plants, at least to fulfill certain functions. However, oil palm, which harbors a second AKT2-type channel, EgKT2-2, may use it for functions requiring weak rectification, although experimental evidence is still required.

Importantly, the activity of EgKT2-1 in 100 mM K⁺ was not sensitive to variations in external pH (Fig. S1 in supplementary data) unlike its counterparts, independently of the rectification, e.g. AKT2 in *Arabidopsis*, ZMK2 in *Zea mays* (Philippart et al. 1999), VFK1 in *Vicia faba* (Ache et al. 2001), PTK2 in *Populus tremula* (Langer et al. 2002), VvK3.1 in *Vitis vinifera* (Nieves-Cordones et al. 2019), OsAKT2 in *Oryza sativa* (Huang et al. 2021) or HvAKT2 in *Hordeum vulgare* (Boscari et al. 2009), where external acidification inhibits their activity. This particularity was so far only observed in oil palm and suggests specific physiological conditions requiring a pH-independent channel activity.

Regarding all identified *Shaker*-like members, another different feature of oil palm K⁺ channels is the absence of an AtKC1-like silent channel subunit, what was also observed in coconut and date palm genomes (Yang et al. 2021). However, lack of certain channel members is not exclusive to palms. Recently, Hao et al., (2025) reported the existence of 7 K⁺ channels in centipedegrass species, divided into groups I, II and V, lacking both, group III (weak rectifiers K_{weak}) and group IV (silent units AtKC1-like), indicating that the number of *Shaker*-type members and functions can vary from one species to another.

Role of EgKT2-1 in oil palm potassium uptake

The results obtained using quantitative RT-qPCR showed *EgKT2-1* to be expressed in all tissues, including source and sink organs, mainly in the meristem, leaves and young fruits, whereas members of other plant species display more specific expression in vascular tissues. Thus, in *Arabidopsis*, AKT2 is expressed in all aerial organs, primarily in phloem tissues in both source and sink organs located throughout the plant (Lacombe et al. 2000). In rice, OsAKT2 is mainly expressed in the phloem vascular tissue of the aerial plant parts (Huang et al. 2021), preferentially in shoots (Tian et al. 2021), and positively regulates salt tolerance and grain yield by mediating K⁺ redistribution. In grapevine, VvK3.1 (AKT2 ortholog) is expressed in the phloem vasculature, the expression increases during grape development and reaches a maximum in ripe berries (Nieves-Cordones et al. 2019).

More precisely, *in situ* hybridization showed that *EgKT2-1* was expressed in the phloem and cortex in roots of young seedlings. Together with the functional characterization of EgKT2-1 mediating large inward K⁺ uptake currents, this expression in young roots suggests K⁺ uptake from the soil and potentially translocation to the stele as observed in *Arabidopsis* for AKT1 (Lagarde et al. 1996).

In barley, HvAKT2 is mainly expressed in mesophyll cells (Boscari et al. 2009), suggesting that HvAKT2 (with HvAKT1) may contribute to K⁺ uptake into mesophyll cells of growing leaf tissue. Moreover, in oilseed rape, Hu et al. (2022) showed that K⁺ availability influences mesophyll structure and coordinates CO₂ and H₂O conductance during leaf expansion. Thus, K⁺ promotes mesophyll conductance for CO₂

and H₂O as well as leaf expansion, thereby accelerating leaf function and growth in a coordinated way. In addition, K⁺ deficiency caused uncoordinated changes in mesophyll cell morphology and chloroplast density, which inhibited mesophyll conductance and lead to a decrease in leaf photosynthesis rates and, consequently, yield. In oil palm, like in barley, *EgKT2-1* is expressed in mesophyll cells, exclusively in palisade parenchyma cells, and no expression was detected either in stomata or in vascular bundles. The chlorophyllous palisade parenchyma, where photosynthesis takes place, located in the upper part of the mesophyll, consists of elongated, tightly packed cells rich in chloroplasts. Its structure optimizes the absorption of light energy. The cells in this tissue produce the plant's energy through photosynthesis. Since, *EgKT2-1* is located in palisade parenchyma cells and considering its functional characteristics contributing to K⁺ uptake into mesophyll cells, we hypothesize that *EgKT2-1* present in young leaves might contribute to K⁺ absorption in palisade parenchyma and could thus plays a role in photosynthesis.

Importantly, in fruits, the gene expression level of *EgKT2-1* is stronger during the early stages of fruit development and the transcripts were detected only in mesocarp cells during these stages (*i.e.* 60 DAA). This expression of *EgKT2-1* in mesocarp cells implies a greater influx of K⁺ into these cells during fruit development, coinciding with the increase in K⁺ content observed before lipid biosynthesis, which takes place around 120 DAA.

Regarding the importance of K⁺ availability in metabolism through its influence on several enzymatic activities, the pyruvate kinase might be specifically important in oil palm. This kinase is involved in the production of pyruvate from phosphoenolpyruvate during the final stages of glycolysis. Pyruvate is the key metabolite linking glycolysis to fatty acid synthesis (via acetyl-CoA production) which are the main components of oil (Mirande-Ney et al. 2019). In the *E. guineensis* mesocarp, the key factors in oil biosynthesis are pyruvate supply and transcriptional regulation of fatty acid synthesis and triglycerides assembly enzymes unlike in other palms such as the date palm (*Phoenix dactylifera*), that store almost exclusively sugars (Bourgis et al. 2011). To form the pyruvate dehydrogenase complex, pyruvate decarboxylase is required, together with dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase. This complex is involved in the *de novo* fatty acid biosynthesis, catalyzing the conversion of pyruvate to acetyl-CoA (Cui and Tcherkez 2021). Pyruvate dehydrogenase is a K⁺-dependent enzyme, as K⁺ is involved in the loop structure between the alpha and B leaflets of the E1 subunit. In addition, Cui et al. (2019), showed that under low K⁺ availability, greenhouse oil palm developed an alternative pathway to produce pyruvate, which suggests high dependency on K⁺.

Despite so far only few reports regarding the effect of K⁺ availability for fruit development and oil biosynthesis in oil palm, an impact on oil yield and composition can be expected during the early stages of palm fruit development. According to Lai and Sheu (1985), pyruvate decarboxylation activity can increase by 5-100% when K⁺ concentrations (100 mM) are higher compared to low K⁺ concentrations (5 mM). More recently, Mirande-Ney et al. (2019), showed that K⁺ availability had a significant effect on mesocarp metabolism, but the composition of oil in mature fruits was not affected.

EgKT2-1 mediates K⁺ transport critical for oil production in oil palm

Commercial oil palm varieties are generally tenera hybrids resulting from crosses between heterotic group A (mainly of Deli origin, dura palms) and group B (mainly of African origin such as La Mé, pisifera palms). Two crosses, C2 and C3, sharing the same genetic background (Deli × La Mé), were used in this study. Under the environmental conditions prevailing in Nigeria and with identical K⁺ fertilizer applications, the C3 progeny was recently reported to produce higher oil yields and more bunches than the C2 progeny (Dassou et al. 2022a, b).

Between 5 and 8 years after planting (YAP), the C3 progeny produced 15.8 t of Fresh Fruit Bunches (FFB) ha⁻¹ year⁻¹ and 4.41 t of crude palm oil (CPO) ha⁻¹ year⁻¹ at mill level, whereas the C2 progeny produced 14.2 t FFB ha⁻¹ year⁻¹ and 3.95 t of CPO ha⁻¹ year⁻¹. At 7.5 YAP, the highest K⁺ content in vegetative organs (rachis and petiole) was observed in the C3 progeny (884 g palm⁻¹) compared to the C2 progeny (648 g palm⁻¹). In contrast, the K⁺ quantity stored in the palm trunk was higher in the C2 progeny (1.68 kg palm⁻¹) than in the C3 progeny (1.37 kg palm⁻¹) (Dassou 2023). These phenotypic differences suggest more efficient K⁺ translocation from the trunk to the aerial organs in the C3 progeny, resulting in greater K⁺ availability for bunch production.

The *in situ* hybridization results provide mechanistic insights into how EgKT2-1 may contribute to enhanced K⁺ translocation and oil production. In roots, *EgKT2-1* transcripts were localized in the root cortex and root phloem of young seedlings, with particularly strong labeling observed in the pericycle, especially in pericycle cells adjacent to the xylem poles. This localization is highly significant, because xylem poles are known to contain passage cells, that facilitate the radial transport of K⁺ from the cortex to the xylem vessels (Peterson and Enstone 1996; Steudle 2001). Thus, EgKT2-1 likely play a key role in K⁺ loading into the xylem, enabling efficient long-distance transport of K⁺ from roots to aerial organs, including leaves and developing fruits.

In leaves, *EgKT2-1* transcripts were specifically detected in the palisade parenchyma cells, whereas no labeling was observed in vascular bundles or stomata. The palisade parenchyma is the primary site of photosynthesis, where K⁺ is known to activate key enzymes such as pyruvate kinase and RuBisCO activase (Cui and Tcherkez, 2021). The presence of EgKT2-1 in these cells suggests that it contributes to K⁺ uptake into the mesophyll, thereby enhancing photosynthetic efficiency and sugar production. This increased availability of photoassimilates in source leaves is likely redirected toward sink organs, such as developing fruits, thereby supporting their growth and oil biosynthesis.

In young fruits, *EgKT2-1* transcripts were exclusively localized in mesocarp cells at 60 DAA, coinciding with the early stages of fruit development when K⁺ accumulation reaches its maximum (Desassis, 1961). This temporal and spatial expression pattern strongly suggests that *EgKT2-1* mediates K⁺ uptake into mesocarp cells, where K⁺ is essential for pyruvate dehydrogenase activity, a critical step in fatty acid biosynthesis (Mirande-Ney et al., 2019). The higher expression of *EgKT2-1* in the C3 progeny, which displays greater oil yield, further supports the hypothesis that *EgKT2-1* enhances K⁺ availability in

mesocarp cells during early fruit development, thereby promoting oil biosynthesis and increasing oil yield.

Taken together, our results indicate that *EgKT2-1* is not only correlated with oil production but is also mechanistically involved in K^+ transport and accumulation in key tissues associated with oil biosynthesis. By facilitating K^+ uptake in roots, leaves, and mesocarp cells, *EgKT2-1* likely contributes to the higher oil yield observed in the C3 progeny, highlighting its potential as a target for improving K^+ use efficiency and oil production in oil palm. As *EgKT2-2*, the other channel of the KT2 family, is not expressed in fruits, it is likely that this channel is not involved in oil formation.

CONCLUSION

In conclusion, the functional characterization of *EgKT2-1*, a *Shaker*-type K^+ channel in oil palm, clearly demonstrated that this channel behaves as an inward rectifier. The physiological roles of this channel likely differ depending on the tissue in which it is expressed, roots, leaves or fruits. In roots, *EgKT2-1* is assumed to be involved in K^+ uptake from the soil solution and its subsequent translocation to the leaves. In leaves, *EgKT2-1* potentially contributes to K^+ uptake into palisade parenchyma cells, thereby likely playing a role in photosynthesis processes. Most notably, *EgKT2-1* appears to facilitate K^+ loading into mesocarp cells during the early developmental stages of the oil palm fruit and therefore suggested to contribute to higher oil and bunch yields. In oil palm, K^+ increases fresh fruit bunch production (Corley and Tinker, 2015), which may be partly explained by the role played of *EgKT2-1* as an inwardly rectifying channel. The correlation between *EgKT2-1* expression levels and oil production in the oil palm crosses described here suggests a possible contribution of this K^+ channel to yield efficiency.

Altogether, *EgKT2-1* plays a role in K^+ uptake in source organs (roots and leaves) and in K^+ transport toward sink organs (fruits). In fruits, *EgKT2-1* is involved in K^+ uptake during early development stages, coinciding with the period of K^+ accumulation required for oil biosynthesis and yield formation. This work provides initial insights into the mechanisms of K^+ transport in oil palm and its impact on oil production and opens new perspectives for research aimed at reducing K^+ fertilizer inputs. *EgKT2-1* thus emerges as a promising target for breeding programs, aiming to develop commercial varieties with improved K^+ use efficiency, thereby reducing dependency on K^+ fertilizer, and promoting more sustainable oil palm cultivation.

Declarations

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AUTHOR CONTRIBUTIONS

H.M. conducted electrophysiological experiments and contributed to supervision and revised the manuscript, S.E. conducted *in situ* hybridizations and RTqPCR experiments and contributed to supervision and revised the manuscript, S.D.Z. supervised the electrophysiological experiments and contributed to conceptualization, supervision, and writing-review and editing, N.B. contributed to supervision and revised the manuscript, J.L.V. contributed to supervision and revised the manuscript, S.B. constructed the phylogenetic tree, J.O. contributed to production data from C2 and C3 genotypes and contributed to supervision and revised the manuscript, R.I. collected and placed tissue material in RNAlater reagent and send to Montpellier, F.J. contributed to supervision and revised the manuscript, I.G. contributed to conceptualization, supervision, and writing-review and editing and T.C. conceived and designed research and contributed to conceptualization, supervision, experiments, writing of the original draft, and writing-editing of the manuscript.

Conflict of interest The authors declare they have no conflict of interest and consented to submitting and publishing this study.

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Figures

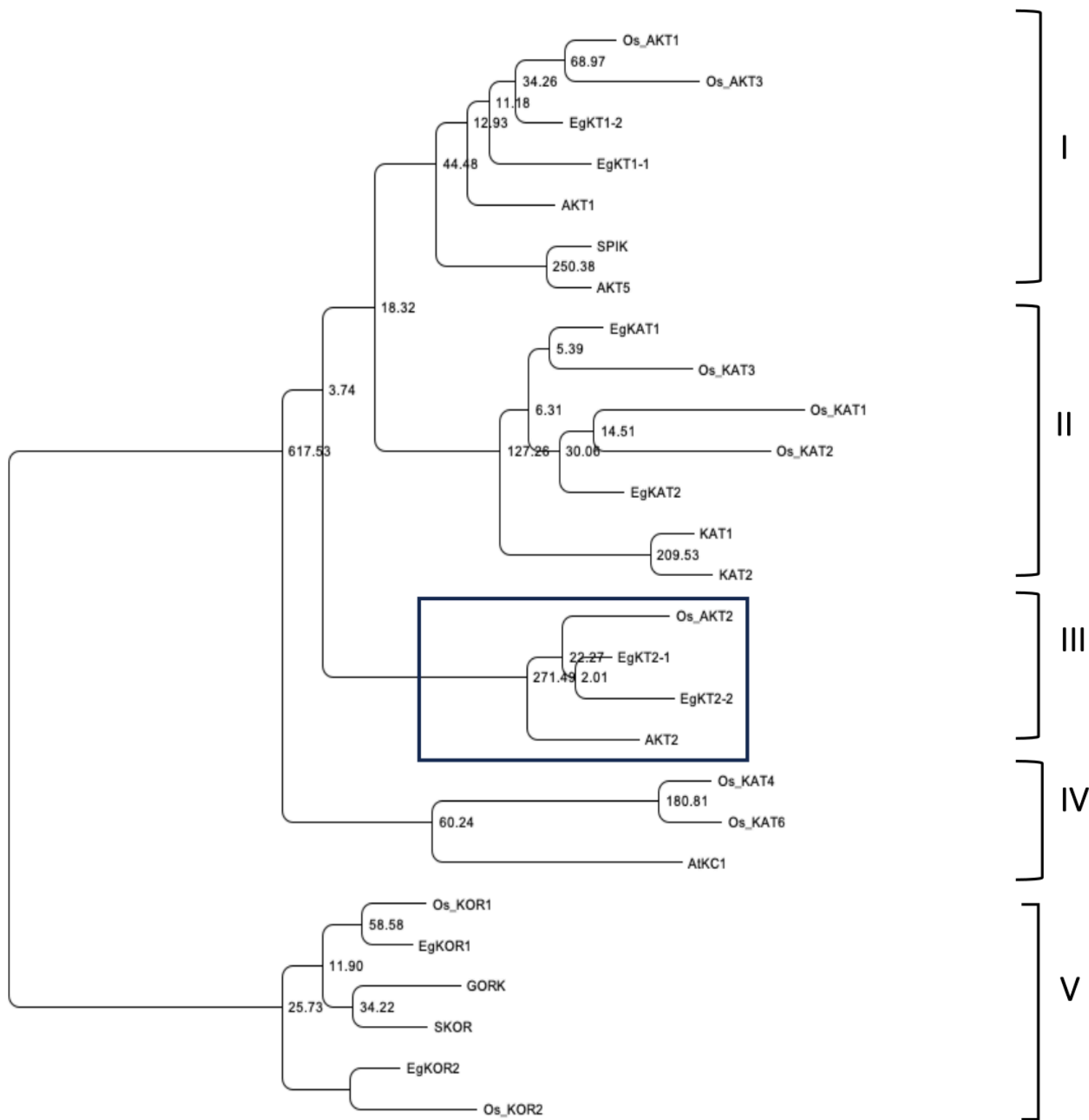
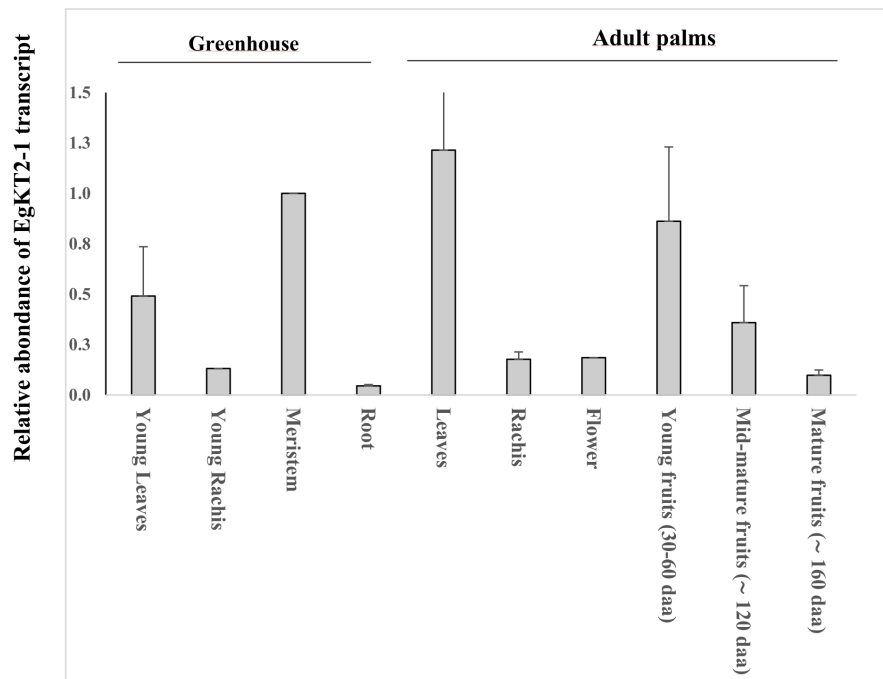
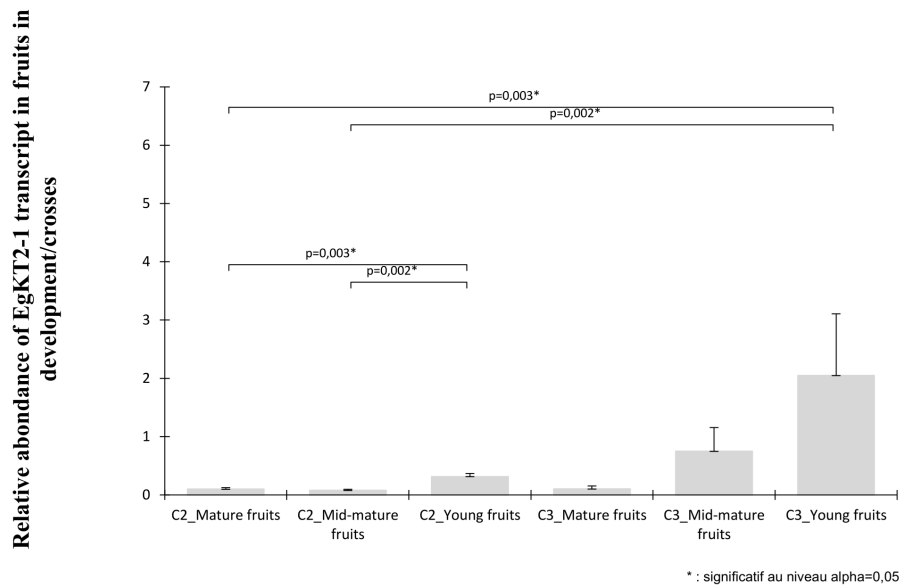


Figure 1

Phylogenetic relationships of *Shaker*-like channels in Arabidopsis, rice and oil palm.



A



B

Figure 2

A: Expression of the *EgKT2-1* channel in oil palm. Relative abundance of *EgKT2-1* transcript levels in oil palm tissues. The expression levels of the *EgKT2-1* gene were investigated by RT-qPCR on total RNA extracted from leaves, rachis, meristem, and roots from young plants as well as from leaves, rachis, flowers and fruits in three developmental stages from adult palms. Analyses of gene expression levels showed that *EgKT2-1* was strongly expressed in leaves, meristem, and young fruits (30 - 60 DAA)

coinciding with the reported increase in K⁺ in the early steps of fruit development. In contrast, *EgKT2-1* was weakly expressed in flowers, roots, rachis, mid-mature fruits (around 120 DAA) and mature fruits (160 DAA).

B: Expression of the EgKT2-1 channel in oil palm in two crosses. Relative abundance of *EgKT2-1* transcript levels on oil palm fruits in two crosses. Expression levels of the *EgKT2-1* gene were analyzed in fruits from two crosses, C2 and C3 (Deli x La Mé) showing that *EgKT2-1* is more highly expressed in C3 fruits than in C2 fruits. Note, the correlation of this expression difference with higher oil and bunch yields of C3 compared to C2 progeny in Nigerian environmental conditions and with the same amount of K⁺ fertilizer, (Dassou et al. 2022a,b).

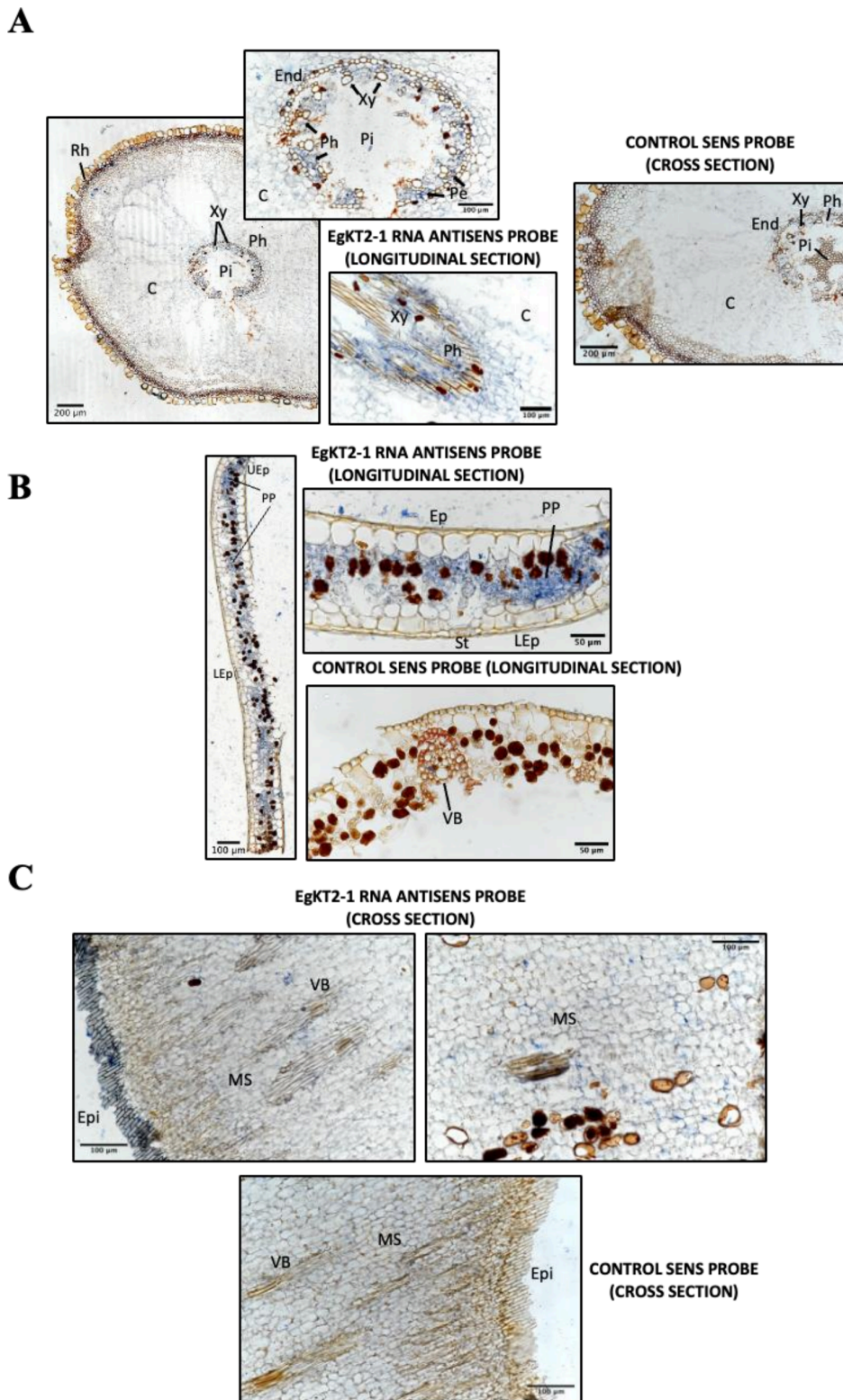


Figure 3

***In situ* hybridization of *EgKT2-1* transcripts in roots (A), young leaves (B) and young fruits (60 DAA) (C).** Cross and longitudinal sections were hybridized with an *EgKT2-1* RNA antisense probe or RNA sense probe. Sections hybridized with the sense probe (negative control) showed no significant signal. A positive blue signal was observed in roots, young leaves and young fruits with the *EgKT2-1* RNA antisense probe. In roots, the signals were detected in the phloem and cortex cells in both sections, in

longitudinal and transversal sections (A). In young leaves, the blue signals were observed in cells of the palisade parenchyma of mesophyll (B). In fruits the signals were detected in the mesocarp cells only in fruits at 60 DAA (C). C, cortex; End, endodermis; Epi, epicarp; LEp, lower epidermis; MS, mesocarpe; Pe, pericycle; PP, palisade parenchyma; Pi, pith; Ph, phloem; St, stomata; Rh, rhizodermis; VB, vascular bundles; UEp, upper epidermis; Xy, xylem; DAA, days after anthesis.

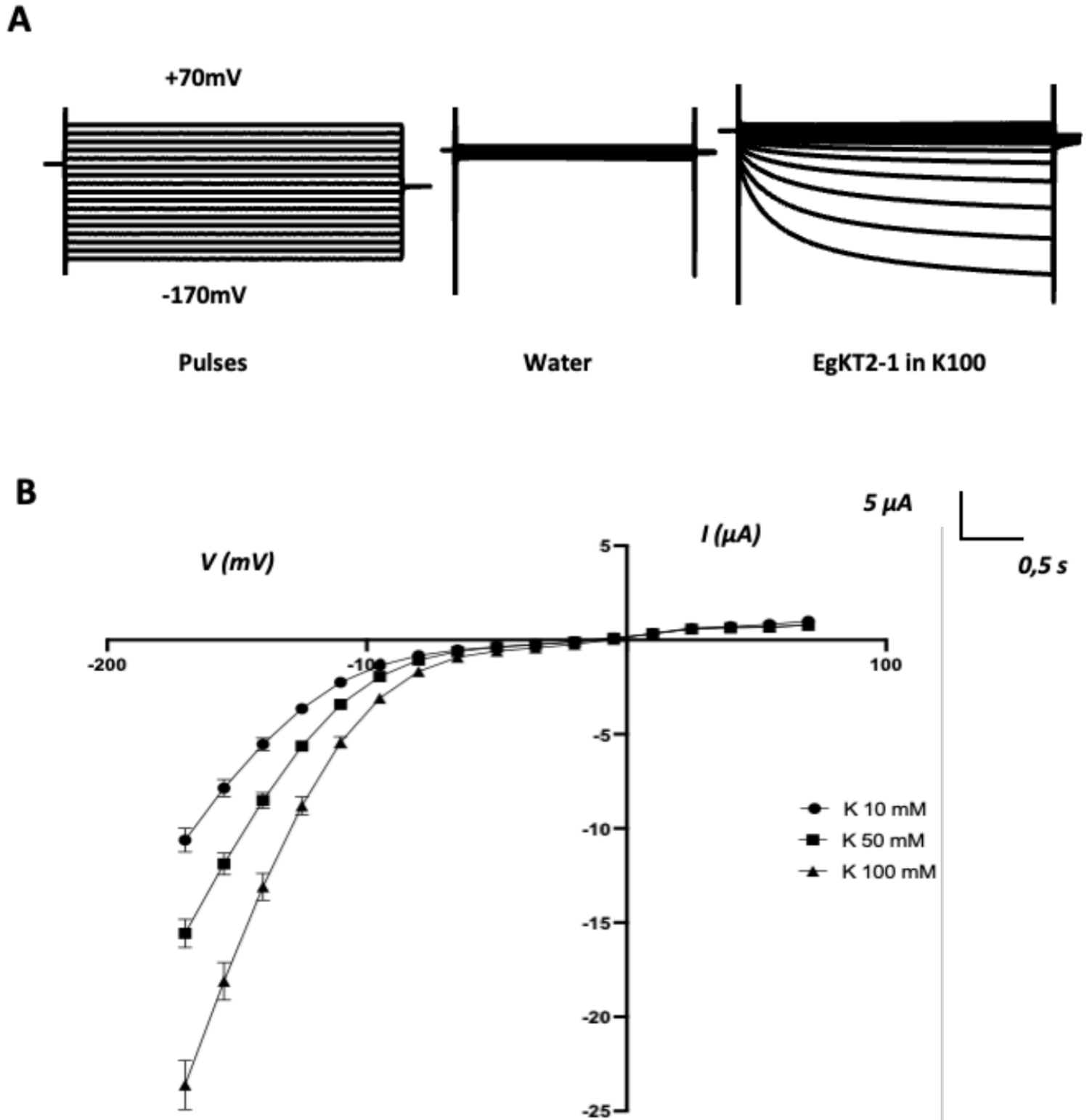
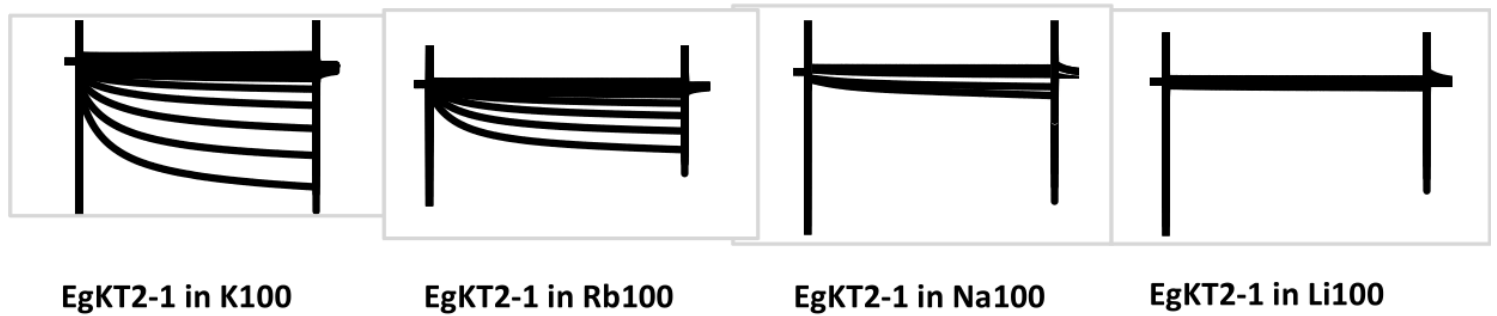


Figure 4

EgKT2-1 is an inwardly rectifying channel mediating inward potassium currents. The functional properties of EgKT2-1 channels were investigated in *Xenopus laevis* oocytes by voltage-clamp analyses. (A) Representative current traces in response to voltage-pulses from +70 to -170 mV for oocytes injected with water as control or with EgKT2-1 cRNA (6 ng) in external bath solution of 100 mM K⁺ gluconate (K100), pH 6,5. (B) Current-voltage curves for mean currents mediated by EgKT2-1 in different external K⁺ concentrations (K10, K50 and K100 mM), pH 6,5 (n=15, $\pm$ SE).

A



B

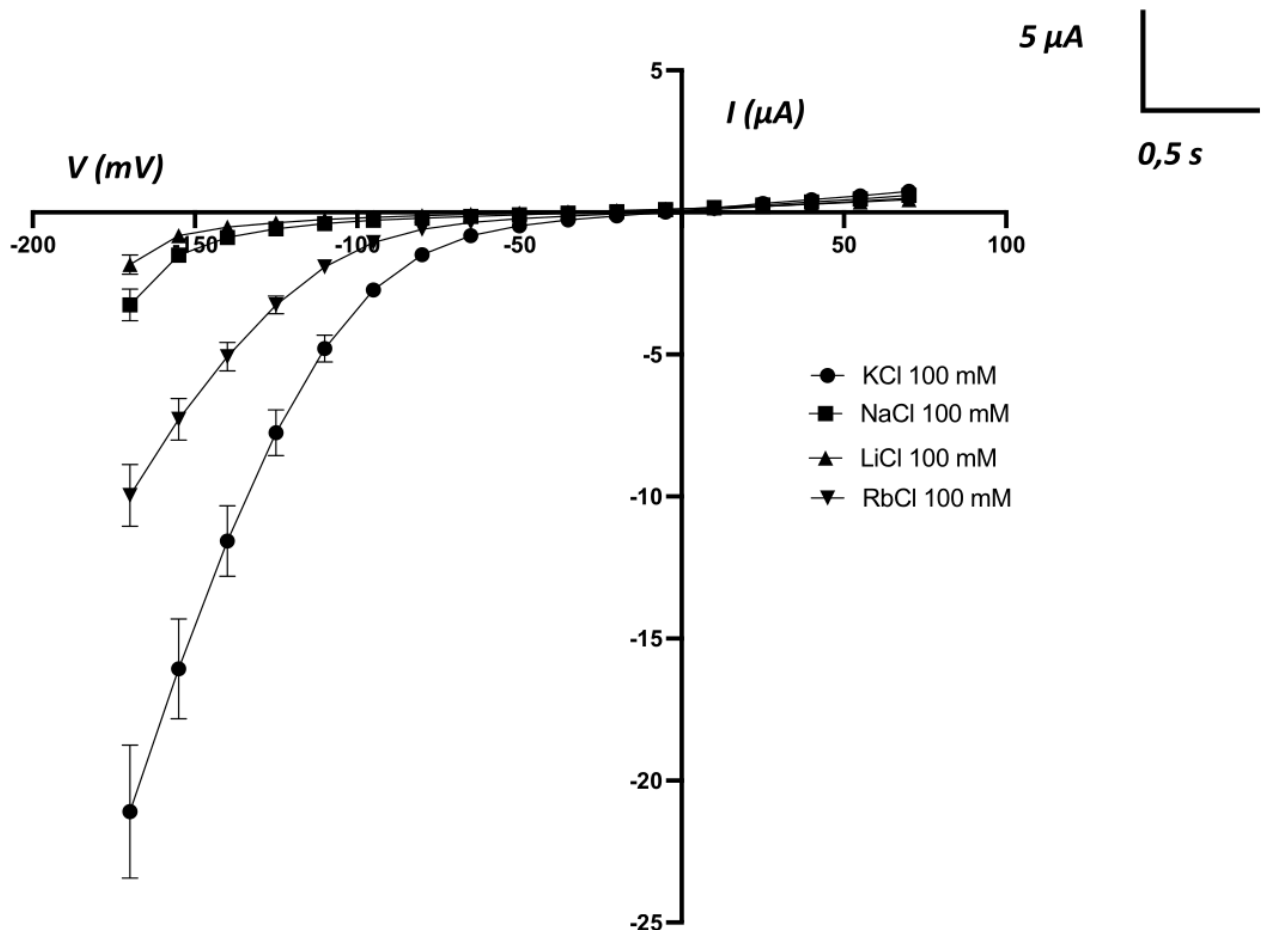


Figure 5

EgKT2-1 is selective for potassium. Permeability of EgKT2-1 for four monovalent cations was tested by electrophysiology in oocytes. (A) Representative current traces in response to voltage-pulses from +70 to -170 mV for oocytes expressing EgKT2-1 in 100 mM cation (K^+ , Rb^+ , Na^+ and Li^+) chloride salt, pH6.5. (B) Current-voltage curves for mean currents mediated by EgKT2-1 in 100 mM K^+ , Rb^+ , Na^+ and Li^+ bath solutions at pH 6,5 (n=11, $\pm$ SE). Note, that only Rb^+ could replace partially K^+ .

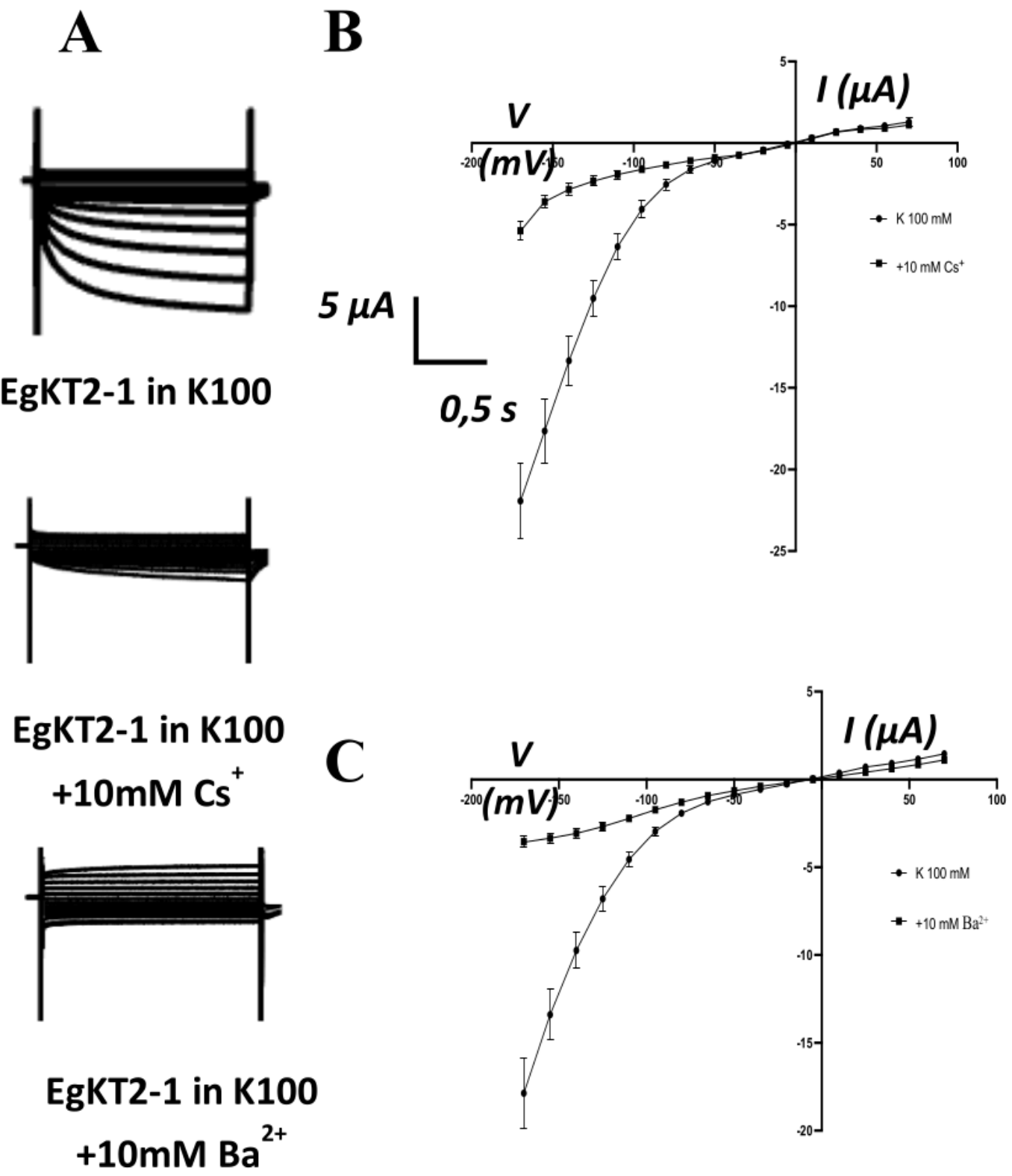


Figure 6

The oil palm channel EgKT2-1 is inhibited by external cesium and barium. (A) Representative current traces in response to voltage-pulses from +70 to -170 mV for oocytes expressing EgKT2-1 in external 100 mM K⁺ solution as control or supplemented with 10 mM of Cs⁺ or Ba²⁺ at pH 6.5. Current-voltage curves are shown for EgKT2-1 currents in 100 mM K⁺ with 10 mM Cs⁺ (B), and with 10 mM Ba²⁺ (C) at pH 6.5 (n=8, ±SE).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureSupplementarydata2VF.pdf](#)