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Melon (*Cucumis Melo* L.) Aquaporins
as Molecular Markers of Resistance to Abiotic Stresses
and Physiopathies

Acuaporinas de Melón (*Cucumis Melo* L.)
como Marcadores Moleculares de Resistencia
a Estreses Abióticos y Fisiopatías

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- **Lopez-Zaplana, A.,** Bárzana, G., Ding, L., Chaumont, F., & Carvajal, M. (2022). Aquaporins involvement in the regulation of melon (*Cucumis melo* L.) fruit cracking under different nutrient (Ca, B and Zn) treatments. *Environmental and Experimental Botany*, 104981.

Apart from the articles included in this thesis, the student Álvaro López Zaplana, during this period of time, has been part of the following articles:

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During the doctorate, the student has presented his results in the following national and international congresses:

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“Las dificultades fortalecen la mente,
como el trabajo lo hace con el cuerpo”
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Melon (*Cucumis melo* L.) aquaporins as molecular markers of resistance to abiotic stresses and physiopathies

Acuaporinas de melón (*Cucumis melo* L.) como marcadores moleculares de resistencia a estreses abióticos y fisiopatías

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Resumen

Resumen

Las acuaporinas son unas proteínas transmembrana que median el transporte de agua y distintos sustratos, siendo inicialmente descubierta su capacidad de transportar agua, posteriormente siendo ampliada a otras moléculas como el glicerol, compuestos nitrogenados como el amonio o la urea, CO_2, H_2O_2 o metaloides como B, Si, As, Se o Sb. Además, dada la importancia del transporte de agua, estas proteínas están muy conservadas y presentes en todos los reinos biológicos.

En plantas, las acuaporinas adquieren un lugar aún más importante, ya que son fundamentales para su correcto desarrollo y necesarias a la hora de adaptarse a condiciones adversas. Así, mientras que en mamíferos hay entre 12 y 15 acuaporinas diferentes, en plantas nos podemos encontrar desde 20 hasta 60, dependiendo de la complejidad del genoma que analicemos. En plantas, estas proteínas se han diversificado, variando su secuencia y con ello su estructura, actividad, localización y capacidad de transporte, encontrándonos 5 familias: PIPs (proteínas intrínsecas de la membrana plasmática), TIPs (proteínas intrínsecas del tonoplasto), NIPs (proteínas intrínsecas homólogas a nodulina-26), SIPs (proteínas intrínsecas pequeñas y básicas) y XIPs (proteínas intrínsecas no caracterizadas).

Además, numerosos estudios demuestran que las acuaporinas están directamente implicadas en la adaptación de las plantas frente a estreses abióticos como pueden ser la salinidad, altas o bajas temperaturas, déficits nutricionales o sequía. Sin embargo, debido a su complejidad y variabilidad, las respuestas entre especies varían enormemente dependiendo del tipo de estrés, su intensidad o la duración del mismo, entre otras variables. Es por ello que se hace necesario estudiar el comportamiento de las acuaporinas en los distintos organismos de interés.

Por otro lado, según la FAO, el melón es una de las frutas más producidas en España, teniendo especial relevancia en la mitad sur del país, siendo los primeros productores a nivel europeo. Sin embargo, debido a que el melón es una planta que suele estar sometida a distintos tipos de estreses debidos al tipo de suelo donde se suele cultivar o a la climatología propia de la época estival (temperaturas elevadas

durante el día, alto contraste entre el día y la noche, posibles lluvias torrenciales), su producción puede disminuir, afectando al rendimiento de los cultivos. Por otro lado, también es bastante usual la aparición de una fisiopatía, el rajado o *cracking*, el cual genera unas aberturas en la corteza, impidiendo la comercialización de los productos y en la cual parecen estar implicadas las acuaporinas. Debido a ello, numerosos grupos han buscado tratamientos foliares que mejoren la incidencia del rajado en distintas frutas, aunque hasta la fecha no se habían descrito tratamientos eficaces en el melón.

Debido a la importancia de este fruto y buscando caracterizar y analizar el comportamiento de las acuaporinas de melón para posteriormente estudiar su implicación en estreses abióticos y la fisiopatía del rajado, esta tesis comenzó identificando las acuaporinas de melón. En el primer artículo titulado "Genome-wide analysis of the aquaporin genes in melón (*Cucumis melo* L.) que corresponde con el capítulo I de esta tesis doctoral, identificamos las 31 acuaporinas del melón (2 PIP1s, 10 PIP2s, 8 TIPs, 8 NIPs, 2 SIPs y 1 XIP) mediante análisis filogenético, las renombramos y eliminamos las duplicaciones y demás errores que había en las bases de datos, indicamos su localización dentro del genoma, longitud de mARN y proteína, peso molecular y punto isoeléctrico, número de hélices transmembrana, al igual que su posible localización subcelular.

Además, discutimos teóricamente qué moléculas eran capaces de transportar y su posible rol dentro de la fisiología de las plantas de melón basándonos en los motivos NPA y variaciones, el filtro de selectividad ar/R y las posiciones de Froger. Por último, se analizaron los niveles de expresión, diseñando cebadores para todas las isoformas, probando su especificidad y asentando las bases para que cualquiera que quiera analizar los niveles de expresión de acuaporinas de melón pueda hacerlo a partir de nuestro artículo.

Respecto a los niveles de expresión, pudimos comprobar que las acuaporinas que tenían los niveles más altos tanto en hoja como en raíz eran *CmPIP1;1*, *CmPIP1;2* y *CmTIP1;1*, presentando esta última la mayor expresión tanto en raíz como en hoja. En un siguiente nivel de expresión estarían *CmPIP2;2*, *CmPIP2;3*,

CmPIP2;6, CmPIP2;10, CmTIP3;1, CmNIP2;1, CmNIP2;2, CmNIP5;1 y *CmNIP5;2*. Por último, y con los niveles más bajos, nos encontramos con *CmPIP2;1, CmPIP2;4, CmPIP2;5, CmPIP2;7, CmPIP2;8, CmPIP2;9, CmTIP1;2, CmTIP1;3, CmTIP2;1, CmTIP4;1, CmTIP5;1, CmNIP4;1, CmNIP6;1, CmNIP7;1, CmSIP1;1* y *CmSIP2;1*. No se detectó expresión de *CmXIP1;1*, pudiendo ser esta acuaporina específica de otros tejidos como fruto o flor, como posteriormente se demostró. Para comprobar los resultados de nuestras RT-qPCRs, comparamos nuestros niveles de expresión con los reportados en un RNAseq previo llevado a cabo en la variedad *Cantalupo* y pudimos encontrar ciertas diferencias, aunque los rangos de expresión en los que se movían las distintas acuaporinas fueron similares, a excepción de *CmPIP2;4, CmTIP2;2* o *CmPIP2;5*.

En el segundo artículo titulado "Relationships between aquaporins gene expression and nutrient concentrations in melon plants (*Cucumis melo* L.) during typical abiotic stresses" que corresponde con el capítulo II de esta tesis doctoral, decidimos analizar la expresión de acuaporinas en plantas de melón sometidas a los estreses abióticos más comunes en nuestra región: estrés por salinidad (proveniente tanto del suelo como de la baja calidad del agua de riego), estrés por altas temperaturas y estrés por déficit de micronutrientes que se puede producir tanto por una mala calidad de suelos como por el efecto lavado de las lluvias torrenciales. El objetivo fue determinar si la respuesta de las acuaporinas y de los nutrientes se asociaría con la que futuramente encontraríamos en el rajado de melón para así, posteriormente, relacionar los resultados. Estos ensayos se realizaron en condiciones controladas de cámara de cultivo.

En primer lugar, se analizaron parámetros fisiológicos fundamentales como el peso fresco y seco, la transpiración, el potencial osmótico en hoja y raíz, el potencial hídrico y el turgor en las hojas, la conductividad hidráulica de la raíz (L_o) y la concentración de los principales cationes que podrían influir debidos a los estreses abióticos o estar relacionados con las acuaporinas (B, Ca, Fe, K, Mg, Mn, Mo, Na, P, S, Si y Zn). De este modo se obtuvo una imagen clara de la respuesta del melón frente al estrés ambiental. Para complementar estos estudios, se

correlacionaron los patrones de expresión de las acuaporinas tanto de raíz como de hoja con los resultados fisiológicos obtenidos, entendiéndose así las implicaciones de las acuaporinas en la respuesta de tolerancia de estas plantas a los distintos estreses testados.

Los parámetros fisiológicos demostraron que el tratamiento que más alteró el estado normal de las plantas fue el estrés nutricional, el cual fue el único estrés propuesto que redujo el peso seco y fresco de la parte aérea, además de aumentar el potencial osmótico en raíces y disminuir la L_o, sugiriendo una reducción del transporte de agua al interior de la planta, que se correlaciona con la disminución de expresión de acuaporinas. Seguido a este tenemos el salino que disminuyó el potencial osmótico de raíz y hojas, el potencial hídrico de hojas y la L_o, al igual que pasaba con el estrés anterior, y aumentó el turgor de las hojas, sin modificar el peso ni de raíz ni de parte aérea, mostrando una alta capacidad para afrontar este tipo de estrés. Opuesto a estos, el estrés por temperatura no modificó ningún parámetro fisiológico demostrando que las plantas de melón fueron tolerantes a este estrés.

En cuanto a los nutrientes, en salinidad, tal y como se esperaba, se pudo observar un incremento en los niveles de Na y un déficit de K (raíces y hojas), que afectaron también a la acumulación de otros nutrientes como Fe, Mn, Si y Zn. En el estrés por déficit de micronutrientes se detecta una clara disminución generalizada en K, Mg, P, S, Fe, Mn, Si y Zn y un llamativo incremento de B (hojas) y Mo (raíz y hojas), asociada la acumulación de este último con un mecanismo de defensa frente a la deficiencia de micronutrientes. En el caso del tratamiento por alta temperatura, pudimos encontrar una disminución de K, P, Fe, Mn y Zn, con un llamativo aumento de B, Mg y Si (en raíces y hojas), asociada su acumulación con la ganancia de resistencia a este tipo de estrés.

Por último, analizando la expresión de acuaporinas obtuvimos más cambios en las raíces que en las hojas. En raíz se obtuvo una disminución generalizada con todos los estreses propuestos en todas las subfamilias de acuaporinas (58 cambios totales de expresión de acuaporinas), principalmente TIPs, NIPs y SIPs, debidos a la señalización del estrés externo al que fueron sometidas. En salinidad solo hubo

un incremento significativo, el de *CmTIP1;1*, la acuaporina que presenta una mayor expresión en las plantas de melón y que media el transporte entre el tonoplasto y el espacio intracelular, consiguiendo una acumulación de agua en el interior celular. En déficit de micronutrientes hubo incrementos en *CmPIP2;2* y *CmNIP5;1*, pudiendo estar esta última relacionada con el transporte de distintos metaloides como el B, microelemento en deficiencia en este estrés y que podría estar favoreciendo su entrada. Por su parte, en alta temperatura hubo incrementos en *CmPIP1;1*, *CmPIP1;2*, *CmPIP2;2* y *CmTIP1;1* relacionadas con el transporte de agua, favoreciendo su transporte al interior de la planta.

Por su parte, en hoja vemos muchos menos cambios frente a raíz (21 frente a los 58) y no hay una respuesta generalizada de disminución. Así podemos observar un incremento de expresión en *CmPIP1;1* y *CmNIP6;1* y disminución en *CmNIP1;1* y *CmNIP2;2* en el estrés salino, incremento en *CmTIP1;3* y *CmNIP1;1* y disminución en *CmPIP2;7, CmPIP2;9, CmTIP1;1, CmTIP2;2, CmTIP4;1, CmNIP7;1, CmSIP1;1* y *CmSIP2;1* en estrés por deficiencia nutricional, e incremento en *CmPIP2;6, CmTIP1;3, CmTIP2;1, CmNIP1;1* y *CmNIP5;1* y disminución en *CmTIP1;1* y *CmNIP7;1* en el estrés por alta temperatura. Todos estos cambios fueron relacionados con la respuesta fisiológica encontrada y estaban relacionados con el contenido en agua y nutrientes específicos.

En el tercer artículo titulado "Aquaporins involvement in the regulation of melón (*Cucumis melo* L.) fruit cracking under different nutrient (Ca, B and Zn) treatments" que corresponde con el capítulo III de esta tesis doctoral, se aplicaron dos tratamientos foliares compuestos por Ca, B y Zn (Ca+B+Zn a partir de ahora) y B y Zn (B+Zn), basándonos en un estudio previo sobre plantas de melón en campo y bibliografía para reducir la incidencia del rajado o *cracking* e identificar los mecanismos implicados. También analizamos los patrones de expresión de acuaporinas en la pulpa de melón, distinguiendo entre melones rajados y no rajados. Por último, se seleccionaron 5 acuaporinas, CmTIP1;3, CmTIP2;2, CmNIP1;1, CmNIP2;2 y CmNIP5;1, por su posible relevancia en el rajado del melón y por su aumento de expresión en fruto con respecto a otros tejidos como raíz y hoja, y

mediante expresión heteróloga en oocitos de *Xenopus laevis* se comprobó *in vivo* el transporte de estas acuaporinas.

Dos meses después del trasplante en campo de las plántulas de melón, tres aplicaciones de estos elementos fueron realizadas una vez por semana (Ca+B+Zn y B+Zn). Una vez realizadas las tres aplicaciones, durante cinco semanas, se contabilizaron los melones totales y los rajados, encontrando disminuciones significativas de la incidencia de los melones a partir de la cuarta y quinta semana con los tratamientos de B+Zn y Ca+B+Zn, respectivamente. Estos resultados corroboran nuestros resultados previos donde la aplicación de micronutrientes y Ca consiguieron reducir el *cracking* que inducimos en campo mediante riegos con exceso de agua y lavado de nutrientes o con agua con alta conductancia, demostrando su implicación y concretamente apuntando al transporte de agua y el balance de nutrientes como posibles causas de la fisiopatía.

Para comprobar si los elementos se habían interiorizado en las frutas, se analizaron B, Ca y Zn, Encontrando incrementos significativos en los melones no rajados con el tratamiento de Ca+B+Zn en todos los elementos. Además, Ca mostró un incremento significativo en los melones rajados de ambos tratamientos con respecto a los melones no rajados del tratamiento control. También se analizó el Si, por su posible implicación con el rajado, mostrando el mismo patrón de incremento en el tratamiento de Ca+B+Zn, señalando al Ca como posible elemento que potencia el transporte de otros elementos.

En cuanto a los patrones de expresión en el fruto, pudimos observar que había ciertas acuaporinas que sus cambios de expresión podrían deberse al estado del melón (rajado o no), como fueron *CmPIP1;1*, *CmPIP1;2*, *CmPIP2;8*, *CmPIP2;10*, *CmTIP1;1*, *CmTIP1;3*, *CmTIP2;2*, *CmTIP4;1*, *CmTIP5;1* y *CmNIP5;1*. Debido a los tratamientos (control, Ca+B+Zn o B+Zn) encontramos significativos los cambios en las acuaporinas *CmPIP1;1*, *CmPIP2;3*, *CmPIP2;4*, *CmPIP2;6*, *CmPIP2;7*, *CmPIP2;8*, *CmPIP2;9*, *CmTIP1;3*, *CmTIP4;1*, *CmNIP1;1*, *CmNIP2;1*, *CmNIP4;1*, *CmNIP5;1*, *CmNIP7;1*, *CmSIP2;1* y *CmXIP1;1*. Por último, los cambios de otras acuaporinas se debieron a la interacción de ambos factores,

CmPIP1;2, CmPIP2;6, CmPIP2;8, CmPIP2;9, CmTIP5;1, CmNIP2;2, CmNIP4;1 y *CmNIP7;1*.

De todas éstas, se destacaron por su relevancia en cuanto a los niveles de expresión y grado de significancia, *CmPIP1;1* y *CmPIP1;2* que podrían estar regulando la entrada de agua a través de la membrana plasmática cuando interaccionan con otras PIP2s. Además, *CmTIP1;1* presentó gran interés ya que es la acuaporina con los niveles de expresión más elevados en melón, tanto en raíz como en hoja y fruto, y está implicada en el intercambio de agua entre el tonoplasto y el espacio intracelular.

Además de estas acuaporinas, CmTIP1;3, CmTIP2;2, CmNIP1;1, CmNIP2;2 y CmNIP5;1 se seleccionaron para estudiar su actividad mediante su expresión en oocitos. Tanto CmNIP2;2 como CmNIP5;1 mostraron actividad de transporte de agua. Además, estas dos fueron las únicas acuaporinas que tenían incrementos significativos en los melones rajados con respecto a los no rajados en la situación control. En cuanto al transporte de B, todas mostraron actividad significativa excepto CmNIP5;1, pero destacó CmNIP2;2 como la acuaporina que mostró mayor actividad. Por último, se determinó la actividad frente a Si, utilizando el análogo Ge, siendo esta vez CmNIP2;2 la única que mostró capacidad de transportarlo.

En conclusión, en esta tesis se han descrito todas las aquaporinas de melón, tanto bioinformáticamente como mediante ensayos *in vivo*. Por otro lado, se han correlacionado sus patrones de expresión tanto en raíz como en hoja, con los cambios fisiológicos que han sufrido las plantas mediante distintos ensayos con los estreses abióticos más frecuentes que sufre el melón en nuestra región (salinidad, deficiencia de micronutrientes y alta temperatura). Además, se ha visto la clara implicación que tiene esta familia de proteínas en una fisiopatía muy común en los frutos del melón, el *cracking*, llegando a estudiar *in vivo* el transporte de algunas aquaporinas seleccionadas. Para finalizar, se encontraron unos tratamientos compuestos por Ca, B y Zn que redujeron significativamente los niveles de rajado en el melón, abriendo nuevas puertas a la solución de este problema y mejorando el rendimiento de los cultivos de melón.

Summary

Summary

Aquaporins are transmembrane proteins that mediate the transport of water and other different substrates, initially discovering their ability to transport only water, later being extended to other molecules such as glycerol, nitrogenous compounds such as ammonium or urea, CO_2, H_2O_2, or metalloids such as B, Si, As, Se, or Sb. In addition, due to their relevance in water transport, these proteins are highly conserved and present in all biological kingdoms.

In plants, aquaporins acquire an even more important place, because they are essential for development processes and adaptation to adverse conditions. Without going any further, while in mammals there are between 12 and 15 different aquaporins, in plants we can find from 20 to 60, depending on the complexity of the genome that we analyse. In plants, these proteins have diversified, changing their sequence and thus their structure, activity, location, and transport capacity, finding us 5 families: PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (nodulin-26-like intrinsic proteins), SIPs (small and basic intrinsic proteins) and XIPs (uncharacterized intrinsic proteins).

In addition, numerous studies show that aquaporins are directly involved in the adaptation of plants to abiotic stresses such as salinity, high or low temperatures, nutritional deficiencies, or drought. However, because of its complexity and variability, the responses between species vary, depending on the type of stress, its intensity, or its duration, among other variables. That is why it is necessary to study the behaviour of aquaporins in the different organisms of interest.

On the other hand, according to the FAO, melon is one of the most produced fruits in Spain, having special relevance in the southern half of the country, being the first producer at the European level. However, since the melon is a plant that is usually subjected to different types of stress due to the type of soil where it is usually grown or the weather typical of the summer season (high temperatures during the day, high contrast between day and night, possible torrential rains), its production can decrease, affecting crop yields. On the other hand, the appearance of

a physiopathy, the cracking, which generates openings in the bark, preventing the marketing of products and in which aquaporins seem to be involved, is also quite common. Due to this, numerous groups have sought foliar treatments that improve the incidence of cracking in different fruits, although to date no effective treatments have been described in melons.

Due to the importance of this fruit and seeking to characterize and analyse the behaviour of melon aquaporins to later study their involvement in abiotic stresses and cracking physiopathy, this thesis began by identifying melon aquaporins. In the first article entitled "Genome-wide analysis of the aquaporin genes in melon (*Cucumis melo* L.) which corresponds to chapter I of this doctoral thesis, we identified the 31 melon aquaporins (2 PIP1s, 10 PIP2s, 8 TIPs, 8 NIPs, 2 SIPs, and 1 XIP) through phylogenetic analysis, we renamed them and eliminated duplications and other errors that were in the databases, we indicated their location within the genome, length of mRNA and protein, molecular weight and isoelectric point, number of transmembrane helices, just as we indicated possible subcellular localization.

In addition, we theoretically discuss which molecules they were capable of transporting and their possible role in melon plant physiology based on NPA motifs and variations, the ar/R selectivity filter, and Froger's positions. Finally, the expression levels were analysed, designing primers for all the isoforms, testing their specificity, and laying the foundations so that anyone who wants to analyse the expression levels of melon aquaporins can do it from our article.

Regarding the expression levels, we were able to verify that the aquaporins that had the highest levels both in the leaf and in the root were *CmPIP1;1*, *CmPIP1;2,* and *CmTIP1;1,* the latter being the highest expression in both the root and the leaf. At a next level of expression would be *CmPIP2;2, CmPIP2;3, CmPIP2;6, CmPIP2;10, CmTIP3;1, CmNIP2;1, CmNIP2;2, CmNIP5;1,* and *CmNIP5;2*. Finally, and with the lowest levels, we find *CmPIP2;1, CmPIP2;4, CmPIP2;5, CmPIP2;7, CmPIP2;8, CmPIP2;9, CmTIP1; 2, CmTIP1;3, CmTIP2;1, CmTIP4;1, CmTIP5;1, CmNIP4;1, CmNIP6;1, CmNIP7;1, CmSIP1;1,* and

CmSIP2;1. Lastly, expression of *CmXIP1;1* was not detected, and this aquaporin could be specific to other tissues such as fruit or flower, as was subsequently verified. To verify the results of our RT-qPCRs, we compared our expression levels with the expression levels reported in a previous RNAseq carried out in the variety *Cantaloupe,* and we were able to verify that differences were found in many genes, although the ranges of expression in which the different aquaporins moved were similar, except for *CmPIP2;4, CmTIP2;2,* or *CmPIP2;5.*

In the second article entitled "Relationships between aquaporins gene expression and nutrient concentrations in melon plants (*Cucumis melo* L.) during typical abiotic stresses" which corresponds to chapter II of this doctoral thesis, we decided to analyse the expression of aquaporins in melon plants subjected to the most common abiotic stresses in our region: stress due to salinity (from both the soil and the low quality of irrigation water), stress due to high temperatures and stress due to micronutrient deficiencies that can be caused by poor soil quality as by the washing effect of torrential rains. These tests were carried out under controlled conditions in a culture chamber. The objective was to determine if the response of the aquaporins and the nutrients correlate with those found in cracked melons.

Firstly, fundamental physiological parameters were analysed, such as fresh and dry weight, transpiration, osmotic potential in leaves and roots, water potential and turgor in leaves, hydraulic conductivity of the root (L_o), and the concentration of main cations that could influence due to abiotic stresses or be related to aquaporins (B, Ca, Fe, K, Mg, Mn, Mo, Na, P, S, Si, and Zn). In this way, a clear picture of the melon's response to environmental stress was obtained. To complement these studies, the expression patterns of both, root and leaf aquaporins, were correlated to the physiological results obtained, thus understanding the implications of aquaporins in the tolerance response of these plants to the different stresses tested.

The physiological parameters showed that the treatment that most altered the normal state of the plants was nutritional stress, which was the only proposed stress that reduced the dry and fresh weight of the aerial part, in addition to increasing the

osmotic potential in roots and decreasing the L_o, suggesting a reduction in the transport of water inside the plant, which correlates with the decrease in expression of aquaporins. Following this, we have the saline that decreased the osmotic potential of the root and leaves, the water potential of the leaves, and the L_o, as it happened with the previous stress, and increased the turgor of the leaves, without modifying the weight of either the root or the aerial part, showing a high capacity to cope with this type of stress. Contrary to these, temperature stress did not modify any physiological parameter, showing that melon plants were tolerant to this stress.

In terms of nutrients, in salinity, as expected, an increase in Na levels and a K deficit (roots and leaves) could be observed, which also affected the accumulation of other nutrients such as Fe, Mn, Si, and Zn. In micronutrient deficit stress, a clear generalized decrease is detected in K, Mg, P, S, Fe, Mn, Si, and Zn, and a striking increase in B (leaves) and Mo (roots and leaves), many times associated its accumulation with a defense mechanism against micronutrient deficiency. In the case of high-temperature treatment, we were able to find a decrease in K, Fe, Mn, and Zn, with an increase in B, Mg, and Si (roots and leaves), their accumulation associated with the gain in resistance to this type of stress.

Finally, analysing the expression of aquaporins, we obtained more changes in the roots than in the leaves. In the root, a generalized decrease was obtained with all the proposed stresses in all the aquaporin subfamilies (51 total changes of aquaporins expression), mainly TIPs, NIPs, and SIPs, due to the signalling of the external stress to which they were subjected. In salinity there was only a significant increase, that of *CmTIP1;1,* the aquaporin that has a higher expression in melon plants and that mediates transport between the tonoplast and the intracellular space, getting an accumulation of water inside the cell. In micronutrient deficiency, there were increases in *CmPIP2;2* and *CmNIP5;1*, the latter possibly being related to the transport of different metalloids such as B, a microelement in deficiency in this stress and which could be favouring its entry. On the other hand, at high-temperature treatment there were increases in *CmPIP1;1, CmPIP1;2, CmPIP2;2,* and

CmTIP1;1, all of them related to water transport, favouring its movement inside the plant.

On the other hand, in the leaf, we see fewer changes compared to the root (21 compared to 58) and the decreased response is not generalized. So, we can see expression increase in C*mPIP1;1* and *CmNIP6;1* and decrease in *CmNIP1;1* and *CmNIP2;2* in salt stress, increase in *CmTIP1;3* and *CmNIP1;1* and decrease in *CmPIP2;7, CmPIP2;9, CmTIP1;1, CmTIP2;2, CmTIP4;1, CmNIP7;1, CmSIP1;1*, and *CmSIP2;1* in stress due to nutritional deficiency, and increase in *CmPIP2;6, CmTIP1;3, CmTIP2;1, CmNIP1;1,* and *CmNIP5;1* and decrease in *CmTIP1;1* and *CmNIP7;1* under high temperature stress. All these changes were associated to the physiological response found and they were related to the content of water and specific nutrients.

In the third article entitled "Aquaporins involvement in the regulation of melon (*Cucumis melo* L.) fruit cracking under different nutrient (Ca, B, and Zn) treatments" which corresponds to chapter III of this doctoral thesis, two foliar treatments were applied composed of Ca, B, and Zn (Ca+B+Zn from now on) and B and Zn (B+Zn), based on a previous study on melon plants in the field and bibliography to reduce the incidence of cracking and identify the mechanisms involved. We also analysed the expression patterns of aquaporins in melon pulp, distinguishing between cracked and uncracked melons. Finally, 5 aquaporins, CmTIP1;3, CmTIP2;2, CmNIP1;1, CmNIP2;2, and CmNIP5;1, were selected for their possible relevance in melon cracking and their increased expression in fruit compared to other tissues. as root and leaf, and through heterologous expression in *Xenopus laevis* oocytes, the transport of these aquaporins was verified *in vivo*.

Two months after transplanting melon seedlings in the field, three applications of these elements were made once a week (Ca+B+Zn and B+Zn). Once the three applications were made, for five weeks, the total and cracked melons were counted, finding significant decreases in the incidence of melons from the fourth and fifth week with the B+Zn treatments and Ca+B+Zn, respectively. These results corroborate our previous results where the application of micronutrients and Ca

managed to reduce the cracking that we induced in the field by irrigation with excess water and nutrient washing or with water with high conductance, demonstrating its implication and specifically pointing to water transport and the nutrient balance as possible causes of physiopathy.

To check if the elements had been internalized in the fruits, B, Ca, and Zn were analysed, finding significant increases in the uncracked melons with the Ca+B+Zn treatment in all the elements. In addition, Ca showed a significant increase in the cracked melons of both treatments with respect to the uncracked melons of the control treatment. Si was also analysed, due to its possible involvement with cracking, showing the same pattern of increase in the Ca+B+Zn treatment, indicating Ca as a possible element that enhances the transport of other elements.

Regarding the expression patterns in the fruit, we were able to find that there were certain aquaporins whose expression changes could be due to the state of the melon (cracked or not), such as *CmPIP1;1*, *CmPIP1;2*, *CmPIP2;8*, *CmPIP2;10*, *CmTIP1;1*, *CmTIP1;3*, *CmTIP2;2*, *CmTIP4;1*, *CmTIP5;1*, and *CmNIP5;1*. Due to the treatments (control, Ca+B+Zn or B+Zn) we found significant aquaporins *CmPIP1;1*, *CmPIP2;3*, *CmPIP2;4*, *CmPIP2;6*, *CmPIP2;7*, *CmPIP2;8*, *CmPIP2;9*, *CmTIP1; 3*, *CmTIP4;1*, *CmNIP1;1*, *CmNIP2;1*, *CmNIP4;1*, *CmNIP5;1*, *CmNIP7;1*, *CmSIP2;1*, and *CmXIP1;1*. Finally, the changes in other aquaporins were due to the interaction of both factors, *CmPIP1;2*, *CmPIP2;6*, *CmPIP2;8*, *CmPIP2;9*, *CmTIP5;1*, *CmNIP2;2*, *CmNIP4;1*, and *CmNIP7;1*.

Of all these, *CmPIP1;1* and *CmPIP1;2* stood out for their relevance in terms of expression levels and degree of significance, which could be regulating the entry of water through the plasma membrane when they interact with other PIP2s. Furthermore, *CmTIP1;1* presented major interest since it is the aquaporin with the highest expression levels in melon root, leaf, and fruit, and it is involved in the exchange of water between the tonoplast and the intracellular space.

In addition to these aquaporins, CmTIP1;3, CmTIP2;2, CmNIP1;1, CmNIP2;2, and CmNIP5;1 were selected to study their activity through their expression in oocytes. Both proteins CmNIP2;2 and CmNIP5;1 showed water

transport activity. Furthermore, these two were the only aquaporins that had significant increases in cracked melons compared to uncracked ones in the control situation. Regarding the transport of B, all showed significant activity except CmNIP5;1, but CmNIP2;2 stood out as the aquaporin that showed the highest activity. Finally, the activity against Si was determined, using the Ge analog, this time being CmNIP2;2 was the only one that showed activity.

In conclusion, in this thesis all melon aquaporins have been described, both bioinformatically and through *in vivo* essays. On the other hand, their expression patterns have been correlated both in the root and in the leaf, with the physiological changes that the plants have suffered through different tests with the most frequent abiotic stresses that melon plants suffer in our region (salinity, deficiency of micronutrients and high temperature). In addition, the clear involvement of this family of proteins in cracking, a very common physiopathy in melon fruits, has been seen, and the transport of some selected aquaporins has been studied *in vivo*. Finally, some treatments composed of Ca, B, and Zn were found that significantly reduced the levels of cracking in melons, opening new doors to solve this problem and improving the yield of melon crops.

List of images, tables and figures

List of tables, images and figures

Table 1. Evolution in the classification of the melon family carried out over the last 80 years. 1: Pitrat et al. 2000 proposed 16 varietas. 2: Robinson and Decker-Walters 1997, proposed 6 groups. 3. Pyzhenkov and Malinina 1994, proposed 16 varietas or convarietas. 4. Grebenscikov 1986, proposed 12 convarietas. 5. Filov 1960, proposed 15 convarietas. 6. Pangalo 1958 proposed 13 species (in genus Melo). 7. Whitaker and Davis 1952, proposed 7 groups. Table adapted from Pitrat et al. 2000.

Figure 1. Mundial production quantities in tons of melons by country in 2020 (Faostats).

Figure 2. Mundial articles production about "*Cucumis melo*" last 10 years. (https://app.dimensions.ai/discover/publication).

Figure 3. Mundial articles production about "Cucumis" and "aquaporins" last 10 years (https://app.dimensions.ai/discover/publication).

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Figure 5. Structure of a monomer of an aquaporin. Own image based in Murata et al., 2000**.**

Figure 6. (A) Structure of aquaporin AtTIP2;1, **(B)** pore hydropathy due to ILE210, HIS88, HIS156, and ARG225, and radius plot across the opening and **(C)** water transport regulation through aquaporin. Figure from Shivaraj et al., 2021 based on Eriksson et al., 2013.

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Figure 8. Model melon "Piel de Sapo" selected for carrying out the tests of this thesis in which cracked melons were involved. Own image.

Figure 9. Possible relations between the abiotic stresses studied and the cracking in melon plants. Own figure.

List of abbreviations

List of abbreviations

A	Leaf area
aa	Amino acid
ar/R motif	Arginine + aromatic residue motif
B+Zn	Foliar treatment with boron and zinc
Ca+B+Zn	Foliar treatment with calcium, boron and zinc
cDNA	Complementary DNA
CmAQPs	*Cucumis melo* L. aquaporins
cRNA	Complementary RNA
CRD	Completed Randomized Design
DNA	Deoxyribonucleic acid
DW	Dry Weight
ER	Endoplasmic Reticulum
FP	Froger's Positions
FP1	Froger's Positions in Loop C
FP2-3	Froger's Positions in Loop E
FP4-5	Froger's Positions in transmembrane helix 6
H2, H5	Helix 2, helix 5
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
iTOL	Interactive Tree Of Life
J_v	Exuded sap flow
L_o	Root hydraulic conductivity
LE1, LE2	Loop E 1 and 2
MIP	Major Intrinsic Proteins
mRNA	Messenger RNA
Mw	Molecular weight
NPA	Asparragine-Proline-Alanine motif
NPT	Asparragine-Proline-Threonine motif
NPS	Asparragine-Proline-Serine motif
NPV	Asparragine-Proline-Valine motif

NIP	Nodulin-26-like Intrinsic Proteins
NJ	Neighbour Joining algorithm
Osm_{in}	Internal osmolarity
Osm_{out}	External osmolarity
PAR	Photosynthetically Active Radiation
PCR	Polymerase Chain Reaction
Pi	Point isoelectric
PIP	Plasma membrane Intrinsic Proteins
P_f	Water permeability coefficient
$\Delta\Psi$	Osmotic potential difference
Ψ_p	Turgor potential
Ψ_μ	Osmotic potential
Ψ_w	Water potential
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
ROS	Reactive Oxygen Species
RPL	Ribosomal Protein L
RT-qPCR	Reverse Transcription quantitative PCR
SIP	Small and basic Intrinsic Proteins
SPI	Serine-Proline-Isoleucine motif
T	Time
TIP	Tonoplast Intrinsic Proteins
USER	Uracil-Specific Excision Reagent (cloning technique)
V_t/ V_0	Relative volume change
V_w	Water molar volume
W_0	Weighed immediately
W_f	Weighed after 2, 4, or 6 hours
XIP	Uncharacterised (X) Intrinsic Proteins
YFP	Yellow Fluorescent Protein

Introduction

1. Introduction

1.1. Botanical description of melon (*Cucumis melo* L.)

Melon (*Cucumis melo* L.) is a eudicot diploid species (2n=24) that belongs to the Cucurbitaceae family, to the *Cucumis* genus. Apart from the melon, this genus also includes cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* L.), pumpkin (*Cucurbita moschata* L.), and courgette (*Cucurbita pepo* L.), all of which are of great economic relevance (Garcia-Mas et al., 2012).

The genus *Cucumis* sp. is divided into two subgenera that separate the African species from the Asian ones. Although there are different classifications of the melon, surely the most accepted one is the classification that divides it into 16 groups, 5 of which are grouped in the *agrestis* subspecies and 11 in the *melo* subspecies (Pitrat et al., 2000) **(Table 1).**

The melon plant is annual and herbaceous, creeping or climbing depending on the growing conditions. Its large root system has rapid development, with many ramifications. Both stems and main leaves are covered with hairy formations and villi, both on the upper and lower sides. The leaves are oval in shape, usually pentagonal and with jagged margins. The flowers are yellow and can be male, female, or hermaphrodite. Pollination is entomophilous, mainly carried out by bees (Handel, 1982; Monforte et al., 2004).

The melon is a species with great genetic variability and is widely cultivated throughout the world. A study from 2010 that sequenced the nuclear and plasmid DNA of 100 accessions of *Cucumis* from different geographical areas (Africa, Australia, and Asia) has suggested that the melon has an Asian origin and that its wild ancestor probably originated in India (Sebastian et al., 2010).

1	2	3	4	5	6	7
cantalupensis	cantalupensis	cantalupa	melo	cantalupa	cantalupa	cantalupensis
reticulatus		melo	ambiguus	rokkiford	ambiguus	reticulatus
adana		europeus	adana		adana	
chandalak		chandalak	chandalak	chandaljak	chandalak	
ameri		ameri	ameri	oestivalis	ameri	
inodorus	inodorus	orientalis	cassaba	orientale	cassaba	inodorus
		rigidus	zard	autumnales+hibermus	zard	
momordica	momordica	momordica	conomon			
chate	flexuosus	adzhur	adzhur	chate	adzhur	
flexuosus		flexuosus	flexuosus	tarra	flexuosus	flexuosus
acidulus		chinensis	conomon	acidulus	conomon	
		indica				
chito	dudaim	dudaim	dudaim	chito		chito
dudaim				dudaim	microcarpus	dudaim
conomon	conomon	conomon	conomon	conomon	conomon	conomon
makuwa		monoclinus		monoclinus	monoclinus	
chinensis		chinensis		acidulus	chinensis	
tibish						

Table 1. Evolution in the classification of the melon family carried out over the last 80 years. **1:** Pitrat et al. 2000 proposed 16 varieties. **2:** Robinson and Decker-Walters 1997, proposed 6 groups. **3.** Pyzhenkov and Malinina 1994, proposed 16 varieties or convarieties. **4.** Grebenscikov 1986, proposed 12 convarieties. **5.** Filov 1960, proposed 15 convarieties or subspecies. **6.** Pangalo 1958 proposed 13 species (in genus *Melo*). **7.** Whitaker and Davis 1952, proposed 7 groups. Table adapted from Pitrat et al. 2000.

1.1.1. Melon characteristics and production

The melon fruit has shapes that range from the perfect spherical to the oval depending on the variety and the cultivar, although it has been discovered that their shape varies depending on their genes, due to their relevance in the construction of the flower or the ovary, as it is the case of the "a" (monoecious) gene and the "p" (pentameric) gene (Périn et al., 2002). The rind is green, white, yellowish, or orange in colour and it has a texture that ranges from smooth to rough with writing. Similarly, the pulp usually has colours that depend on the cultivar and the variety, highlighting the colours green, yellow, and orange.

The melon can ripen climatically or non-climatically, depending on the variety. Climacteric ripening is characterized by an increase in the respiration rate and is initiated by a peak in ethylene concentration. Non-climacteric ripening, on the other hand, is produced by a continuous decrease in the respiration rate and a reduction in ethylene production (Moore et al., 2002). This process can be characterized by the identification of some Quantitative Trail Locus (QTLs) responsible for this character along with the location of firmness genes in the fruit in introgression lines (NILs), which results in this species being proposed as one of the main model species to understand the genetic and molecular regulation of plants in the fruit ripening process (Moreno et al., 2008).

Melon consumption is widely distributed throughout the world and the year. At a nutritional level, it has numerous benefits for being rich in numerous minerals and vitamins such as folic acid, vitamin A and vitamin C (Kolayli et al., 2010). In addition, the pulp is rich in other bioactive compounds like pectins (Güzel and Akpınar, 2019), oils (Alexandra Silva et al., 2019), or even hydrogen-type fuels (Turhal et al., 2019). On the other hand, and following the current trends of taking as much advantage as possible and generating the minimum possible waste, melon processing waste, such as seeds and peels, can be used as a source of polyphenols, carotenoids, fibers, or oils (Gómez-García et al., 2020).

The melon is grown in dry tropical and subtropical areas. Most of the melon cultivation takes place in an open field, usually protected under low tunnels until flowering initiation, covered with polyethylene plastic either to extend the growing season or as a protective barrier against insects or other viral vectors for the late crop, increasing the production (Ekinci and Dursun, 2009; Iapichino et al., 2014). In Spain, it is grown seasonally in spring and summer, depending on the locations and the weather associated with them, mainly in Andalusia, the Region of Murcia, and Castilla-La Mancha. According to FAO data, in 2020, China was the world's leading producer with 13838234 tons, followed by Turkey with 1724856 tons and India with 1330000 tons. Spain ranks in the eleventh position with a production of 610980 tons per year and 18520 ha harvested, being the first country in terms of production and ha harvested in Europe **(Figure 1)** and demonstrating the importance of melon cultivation in our country (fao.org/faostats/).

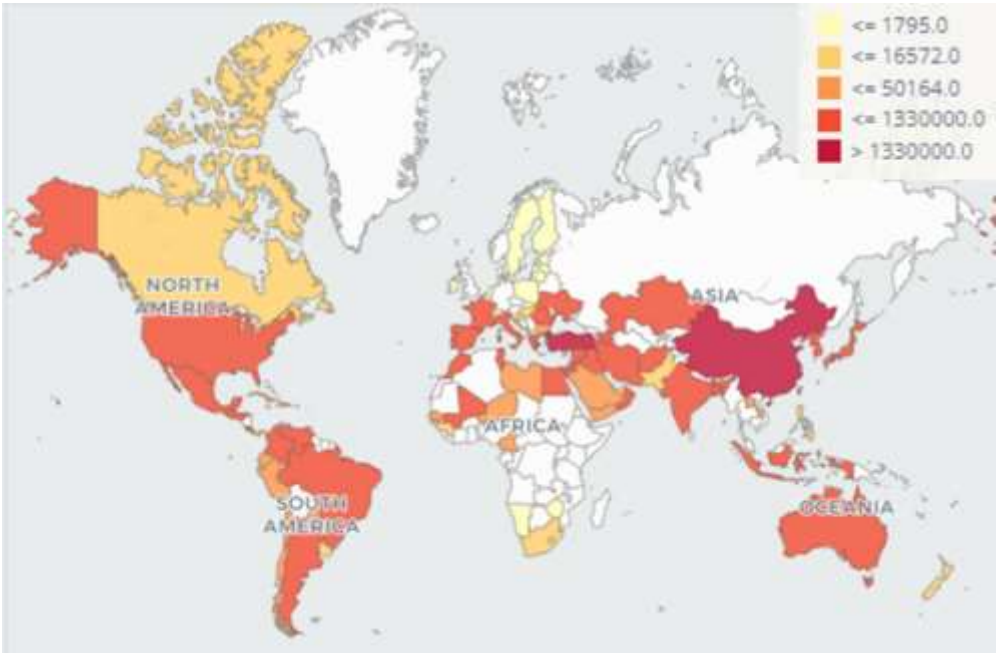

Figure 1. Mundial production quantities in tons of melons by country in 2020 (Faostats).

1.1.2. Scientific production related to *C. melo* L.

In 2009, the project called MELONOMICS was created with the objective of getting an approximation of the genome sequence of melons. Finally, in 2012 the melon genome was encoded and published. It has been possible to assemble more than 84% of the genome containing 27,427 genes (Garcia-Mas et al., 2012). In recent years, the number of melon-related publications has increased, proving a clear upward trend in recent years **(Figure 2)**.

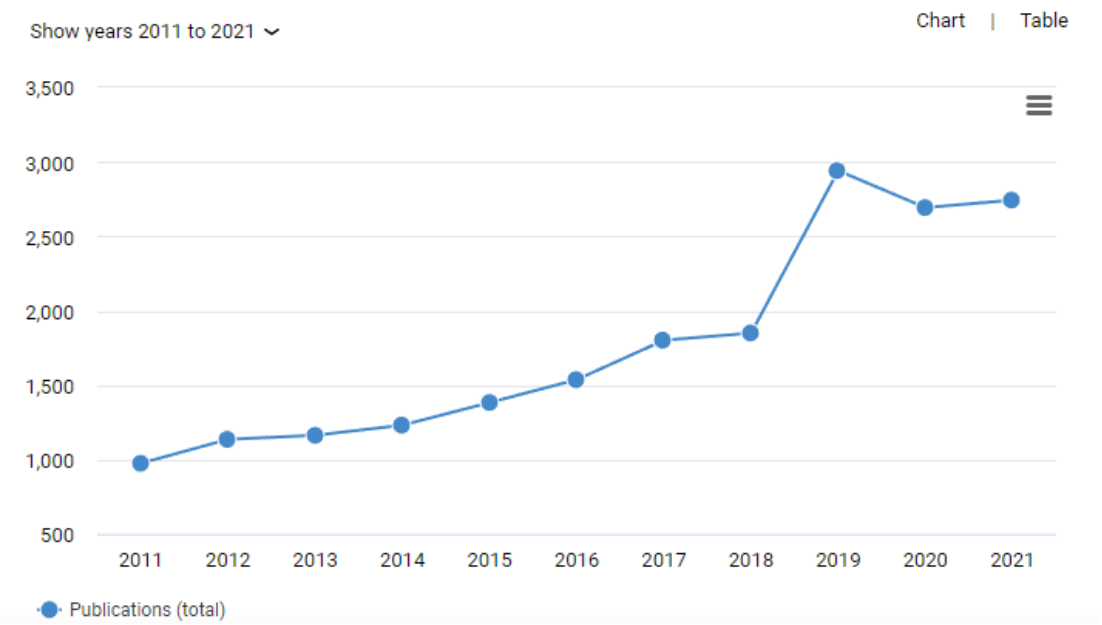

Figure 2. Mundial articles production about "*Cucumis melo*" last 10 years. (https://app.dimensions.ai/discover/publication).

Since that moment, most of their genes have been described, although there were some genes like aquaporins which were neither well described nor studied. However, other related plants such as cucumber (Shi et al., 2015) or watermelon (Zhou et al., 2019) have their aquaporins well described recently. Aquaporins in melon plants play a fundamental role in their development since they need large amounts of water for their proper growth, mainly in the last stages of fruit development (Preciado et al., 2018).

As it can be seen, the trend of articles that include the term "*Cucumis*" and "aquaporins" is also growing, especially since 2019 **(Figure 3)**. However, it was not

until the following year that our group described and named the aquaporins present in melon (Lopez-Zaplana et al., 2020b).

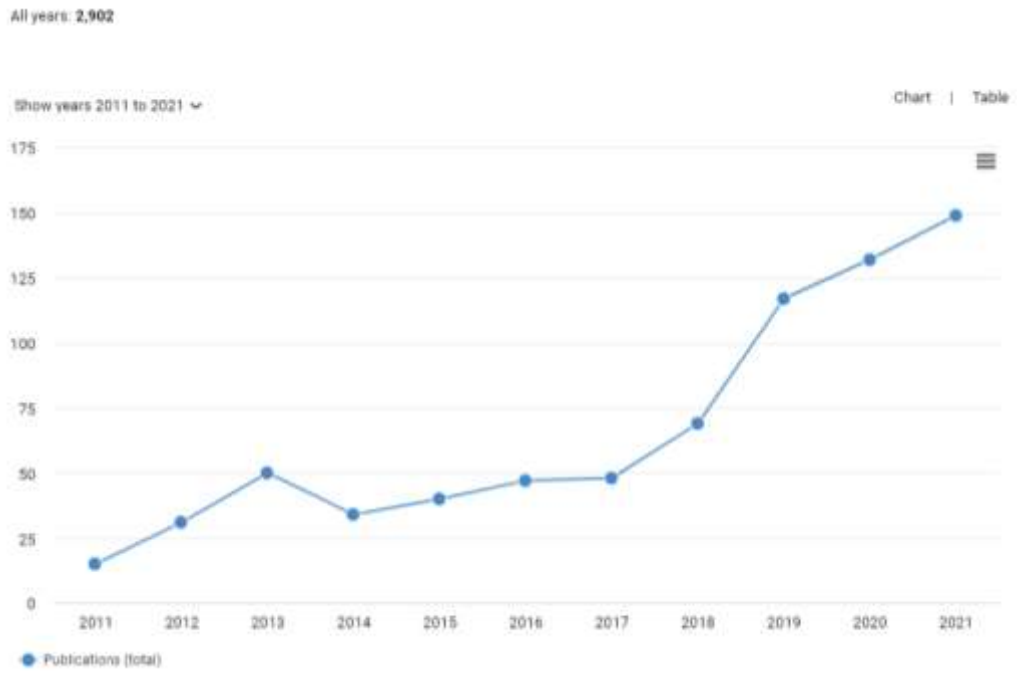

Figure 3. Mundial articles production about "Cucumis" and "aquaporins" last 10 years (https://app.dimensions.ai/discover/publication).

Furthermore, melon plants are typically cultivated in high-temperature regions where they are usually exposed to abiotic stresses related to water deficiency, such as salinity or high temperatures (López-Ortega et al., 2016). At that point, a study regarding the interaction among aquaporins, water, and abiotic stresses related to a physiopathy that decreases the production of melon fruits proves the importance of this thesis.

1.2. Water transport and plants

Water is the main component of living beings, representing around 90% of their cell content. In plants, water is also the main constituent, reaching more than 70% of the fresh weight in non-woody plants and more than 50% in woody plants, although its content varies depending on their physiological state, metabolic activity, and tissue (Ruiz-Lozano et al., 2012).

The importance of water is acting as a solvent that allows the transport of other atoms in the form of gasses, minerals, or ions (Slatyer, 1960), being involved in numerous vital processes for plants such as photosynthesis, maintaining cell turgor, or controlling the opening and closing of the stomata (Chaplin, 2006). However, most of the water contained in plants is lost in the transpiration process, through the opening of stomata, and it is exchanged for the CO_2 necessary to carry out photosynthesis (Larcher, 1995; Rosen et al., 1960).

The uptake of water from the soil is crucial for vascular plants. The movement of water is driven by a potential gradient (Ɗ Ψ), with water moving from regions where the potential is higher to those where it is lower. During transpiration, water evaporation through stomata makes the leaf Ψ lower and produces movement from the xylem towards the leaf surface. This sucking force creates tension in the xylem vessels and drains the soil through the roots (Scharwies and Dinneny, 2019).

Regarding water transport through plant tissues, there are two different pathways involved: the apoplastic pathway where the water goes around the protoplasts, and the cell-to-cell pathway, composed of the symplastic pathway through the plasmodesmata and the transcellular path across the cell membranes (Steudle and Peterson, 1998) (**Figure 4)**.

In order to maintain the high values of water flux, it became evident that transport could not occur by simple diffusion through the lipid bilayer (Zimmermann et al., 1969) and this fact indicates the participation of some transport proteins that facilitated the passage of water across membranes. This fact led to the discovery of aquaporins (Preston and Agre, 1991).

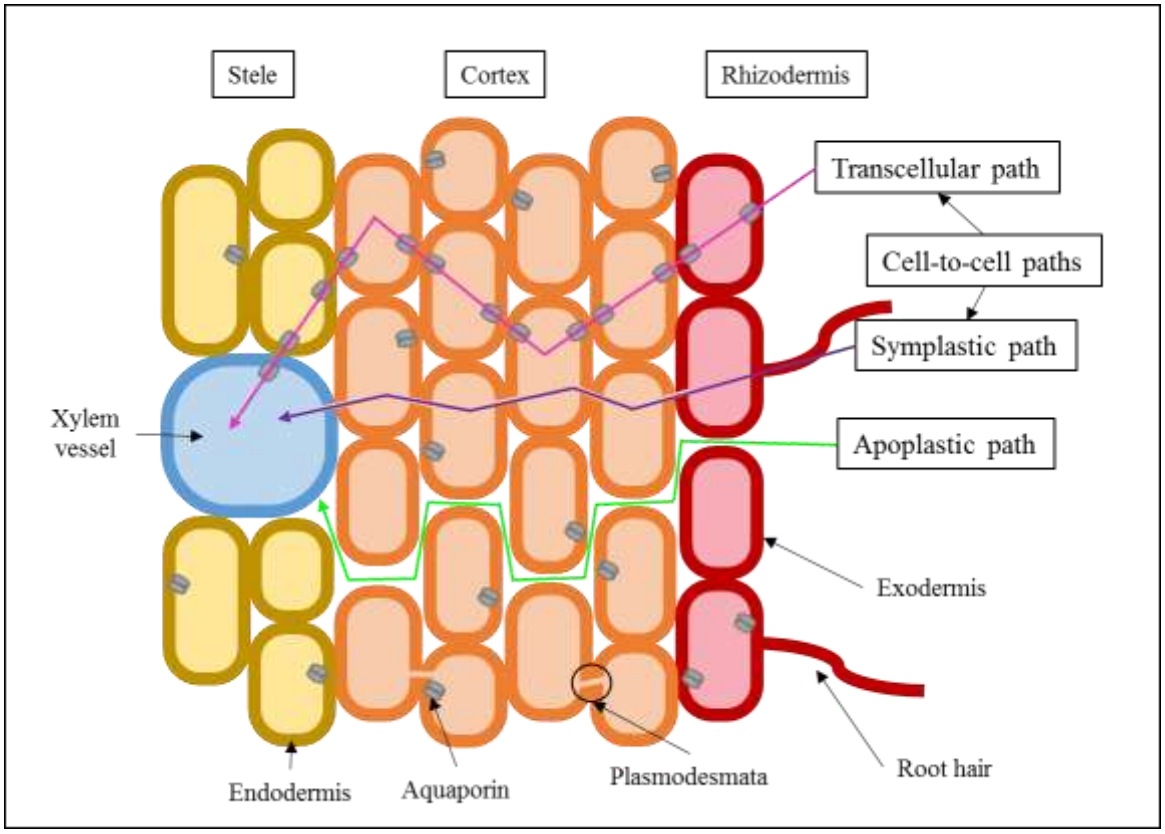

Figure 4. Transport pathways in roots. Own image based in Steudle, 2000.

1.3. Aquaporins

Aquaporins are transmembrane proteins belonging to the MIPs family (major intrinsic protein) that facilitate rapid and selective bidirectional water and low-molecular-mass solutes in response to osmotic gradients (Luang and Hrmova, 2017). They constitute an ancient family and are present in the three kingdoms of life (Eukarya, Bacteria, and Archaea) pointing to their essential role in basal life functions (Laloux et al., 2018).

The discovery of aquaporins was casual. In 1987, a group of researchers found a polypeptide fragment in erythrocytes (Agre et al., 1987) that in 1991 was described as an integral membrane protein canal of 28 KDa (Preston and Agre, 1991). A year before, an intrinsic tonoplast protein had been described, suggesting that this channel-type protein could be involved in the transport of the tonoplast (Johnson et al., 1990). The first activity as the first water transport channel in 1992

(Preston et al., 1992) marked the beginning of the interest in studying new lines of research, taking into account the processes in which these proteins were involved.

However, the term aquaporin was not coined until 1993 by Peter Agre and colleagues (Agre et al., 1993) when they suggested that major intrinsic proteins (MIPs) can mediate the movement of water in the direction of an osmotic gradient (Agre et al., 1993). That same year, the group of Marteen Chrispeels discovered in other higher organisms, such as plants, that tonoplast aquaporin AtTIP1;1 of Arabidopsis (*Arabidopsis thaliana* L.) had a fundamental role in their homeostasis and water transport by means of its expression in *Xenopus laevis* L. oocytes and cell-swelling experiments in hypoosmotic medium (Maurel et al., 1993).

Nowadays, it is known that the growth and development of plants are totally dependent on water, the role of aquaporins in the physiological states of the plants and their water relations is clear and there is a widespread consensus in the scientific community. The water diffusion through the membranes is facilitated by aquaporins, modifying their expression patterns or their activity through their interaction with other proteins or posttranslational modifications (Chaumont and Tyerman, 2014).

Despite their initial discovery as water channels and their importance in regulating water flow in plants, keeping the cellular water homeostasis (Hachez et al., 2006), there are numerous small uncharted solutes that some aquaporins are capable of transporting. Some molecules and elements significant for plants that aquaporins are able to transport are CO_2 (Kaldenhoff, 2012), H_2O_2 (Bienert and Chaumont, 2014), ammonium (Bertl and Kaldenhoff, 2007), glycerol (Dean et al., 1999), urea (Gerbeau et al., 1999) or metalloids such as boron (B) (Takano et al., 2006), silicon (Si) (Chiba et al., 2009), selenium (Se) (Zhao et al., 2010), arsenic (As) or antimony (Sb) (Bienert et al., 2008b; Bienert and Jahn, 2010). Apart from the elements and molecules already described, some others that are also capable of passing through aquaporins have been proposed in recent years. Some of these are sodium (Na), potassium (K) (Tran et al., 2020), lithium (Li), cesium (Cs), rubidium (Rb), nickel (Ni), or copper (Cu) (Noronha et al., 2016; Tyerman et al., 2021).

All these discoveries point to the implication of aquaporins in plant metabolism, nutrition, or signalling processes, increasing the complexity and making aquaporins multifunctional channels with different roles not only in water transport.

1.3.1. Aquaporin subfamilies and function

In contrast to mammals, where 12 to 15 different aquaporins are identified (Laloux et al., 2018), a large number of new aquaporin genes are discovered in plants every year. Between 20 to 60 different aquaporins are revealed in flowering plants, being grouped into five subclasses depending on their subcellular location, function, or phylogeny (Chaumont et al., 2001; Jang et al., 2004; Johanson et al., 2001; Sakurai et al., 2005). The main subfamilies of aquaporins discovered in plants are plasma membrane intrinsic proteins (PIPs) (Kammerloher et al., 1994), tonoplast intrinsic proteins (TIPs) (Johnson et al., 1990), nodulin-26-like intrinsic proteins (NIPs) (Heymann and Engel, 1999), small and basic intrinsic proteins (SIPs) (Johanson and Gustavsson, 2002), and uncharacterised intrinsic proteins (XIPs) (Danielson and Johanson, 2008).

The first plant PIPs genes were identified in *A. thaliana* L. root, using immunoselection and COS cells (Kammerloher et al., 1994). In that article, the two subfamilies of paralogues were discovered, PIP1s and PIP2s, along with their water transport activity. After this discovery, several studies point out that PIP1s act as PIP2s modulators of water activity channels, and they are regulated by posttranslational mechanism and pH (Scochera et al., 2022; Yaneff et al., 2015), but they could also transport other solutes like H_2O_2 (Hooijmaijers et al., 2012) or CO_2 (Uehleln et al., 2003).

PIP1 and PIP2 have more than 80% of sequence identity, whereas they usually have different activities and modifications that affect their functionality. PIP2 C-terminal regions are around 18 to 23 residues longer than PIP1 while the N-terminal region of PIP1 is longer than PIP2 (Scochera et al., 2022).

Although the use of the two subfamilies, PIP1s and PIP2s, is widespread and accepted, some studies redefined the PIP family organization in 3 different orthologous gene clusters for flowering plant species (Soto et al., 2012). In addition to the plasma membrane, PIPs can be localized on chloroplast cell envelopes and thylakoids, denoting the implication of water, CO_2, or H_2O_2 transport in the stroma for the photosynthesis pathway (Ferro et al., 2010; Uehlein et al., 2008).

TIPs were the first aquaporins discovered (Johnson et al., 1990) in plants. TIPs were named according to their main position in plant cells, the tonoplast, where they have the fundamental function of maintaining the cell turgor pressure (Leitão et al., 2014), even when some of them have been found in the endoplasmic reticulum (ER) (Porcel et al., 2018) but also in the chloroplast cell envelop and thylakoids membranes (Ferro et al., 2003; Uehlein et al., 2008). Along with their water activity channels, it is found some TIPs that can transport other compounds or elements like nitrogen (N) in the form of urea or ammonia (Dynowski et al., 2008).

NIPs aquaporins were named like that because of their similarity with nodulin-26 protein (Wallace et al., 2006). What is more, the first time they were named was in a classification where 2 clusters of aquaporins were described: AQP (aquaporin family) cluster and GLP (glycerol facilitator-like protein family) cluster where different aquaporins of animals, bacterial, yeast, and plant aquaporins were classified depending on their ability to transport water or glycerol (Heymann and Engel, 1999; Wallace et al., 2002). After this classification, NIPs were subdivided into 3 subgroups, according to their residues of the ar/R filter (arginine, aromatic residue) (Mitani et al., 2008). NIPs expression is usually lower than other MIPs like PIPs or TIPs and they can exhibit cell or tissue-specific expression that is subject to spatial or temporal regulation (Wallace et al., 2006). Lately, they are also known as metalloid-porins, due to their capability to transport metalloids like Si, Ge, Se, As, or Sb, along with water (Bienert et al., 2008a; Pommerrenig et al., 2015; Sabir et al., 2020).

When the popularity of aquaporins increased, more researchers tried to find all their families. After several years, SIPs were discovered in 2002 (Johanson and

Gustavsson, 2002), and they were named like that as a result of their shorter sequence. This subfamily normally has fewer members, and they can be located in the ER. Usually, SIPs can transport water (Ishikawa et al., 2005)

XIPs were the most recent aquaporins discovered in plants owing to their difficult localization patterns or their low expression (Danielson and Johanson, 2008), although now we know that they are expressed in entire cell membranes like PIPs or NIPs (Shivaraj et al., 2021). They are known to be multifunctional, with several molecules that they are able to transport like water, metalloids, and reactive oxygen species (ROS) (Bienert et al., 2011; Lopez et al., 2012), even glycerol, Ni, and Cu (Noronha et al., 2016). However, they are not present in some higher plant families like some monocots or *Brassicaceae* (Deshmukh et al., 2015).

1.3.2. Aquaporin structure and transport.

Aquaporins are intrinsic membrane proteins with a molecular weight of 21 to 35 KDa. They consist of six transmembrane α-helix (H1-H6) connected by five loops (LA-LE) (Gonen and Walz, 2006; Murata et al., 2000) **(Figure 5)**. The N-terminal and the C-terminal are located in the cytoplasm face and, therefore, they are involved in each monomer activity, modifying their positioning depending on other posttranslational modifications (Eriksson et al., 2013). This symmetrical structure suggests a possible duplication and inversion of a half-size gene that could codify three transmembrane α-helix motifs (Pao et al., 1991).

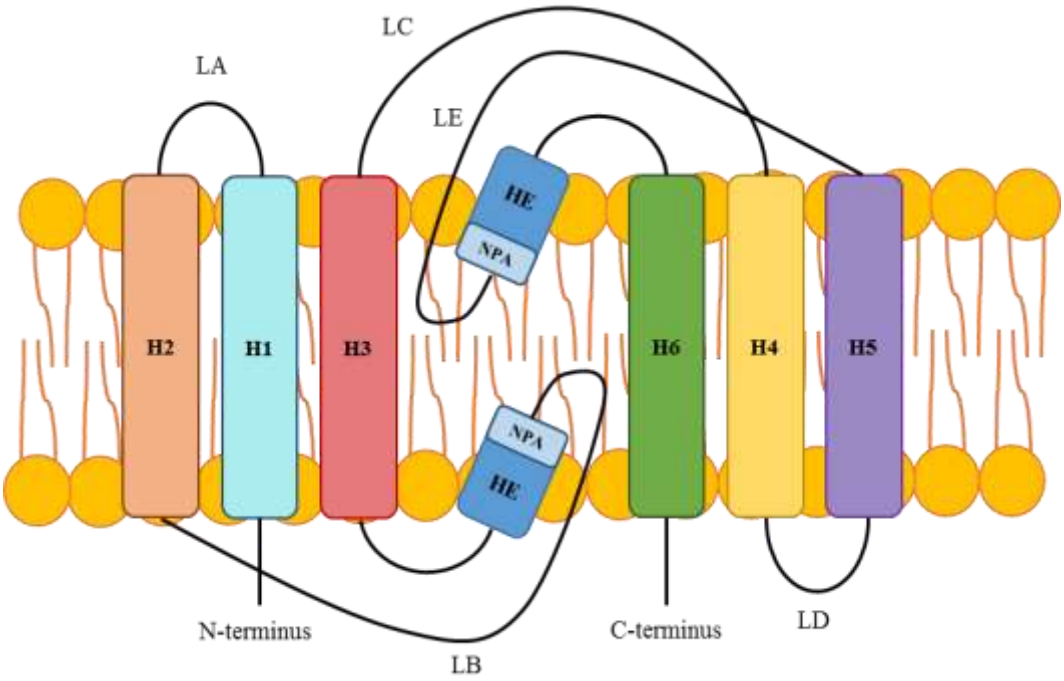

Figure 5. Structure of a monomer of an aquaporin. Own image based in Murata et al., 2000**.**

The two loops LB and LE form two small α-helices (HE) that are inserted into the membrane, in the center of each monomer because of the NPA motif, named after its characteristic asparagine, proline, and alanine residues. This NPA motif is highly conserved in PIPs and TIPs and some variations of it can be found in NIPs, SIPs, and XIPs (Beitz et al., 2006). These motifs serve as a barrier that obstructs the passage of protons when the protein is folded, acting as a filter (Murata et al., 2000). Hydrogen bonding in the asparagine residue of each loop keeps the correct positioning of NPA motifs inside the membrane (Eriksson et al., 2013).

In addition to these two NPA motifs, is the ar/R motif, close to the pore on the extracytosolic face. Both of them are responsible for the characteristic selectivity of transport of each aquaporin according to its pore size and hydrophobicity (Bansal and Sankararamakrishnan, 2007; Wallace and Roberts, 2004).

The majority of the pores are lined by main-chain carbonyl oxygens. These oxygens can act like hydrogens receptors from solutes that balance the energy required to isolate one single molecule of water, breaking the hydrogen bonds of the solution (Eriksson et al., 2013) **(Figure 6)**.

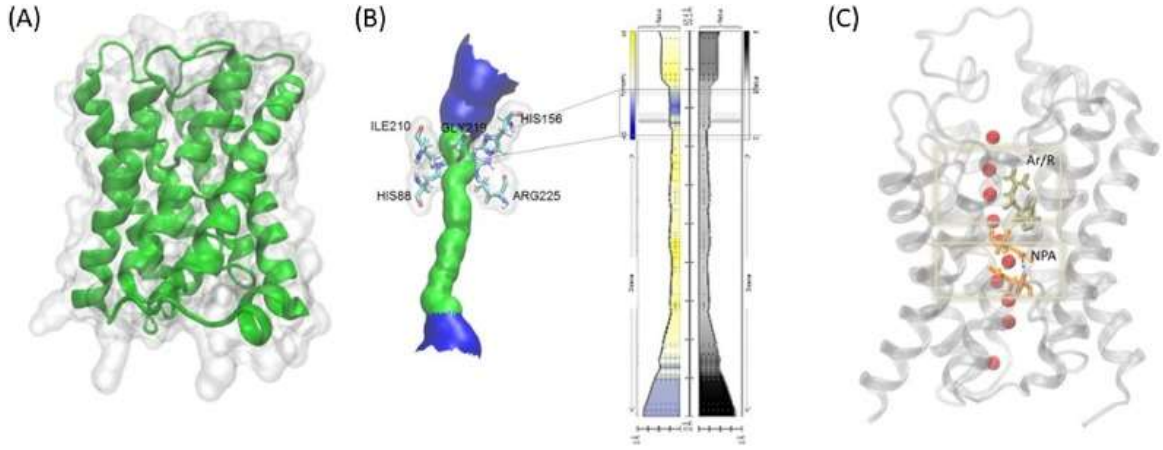

Figure 6. (A) Structure of aquaporin AtTIP2;1, **(B)** pore hydropathy due to Ile210, His88, His156, and Arg225, and radius plot across the opening and **(C)** water transport regulation through aquaporin. Figure from Shivaraj et al., 2021 based on Eriksson et al., 2013.

The unveiling of the aquaporin structure has allowed a deeper understanding of aquaporin transport activity. The first time an aquaporin was crystallized was in 2006 when SoPIP2;1 structure was solved, which also allowed the discovery of the gating system that was associated with changes in pH (Törnroth-Horsefield et al., 2006).

This posttranslational modification changed the conformation of the aquaporins, pointing to the high importance of loops to activate or inhibit the transport across the aquaporin monomers. The major differences between the open and closed conformation were found in loop D, which was 4 to 7 residues longer than other homologs. In the closed conformation, this loop folds under the aquaporin, preventing the pore access to the cytosol. Moreover, 4 residues, Leu197, His99, Val104, and Leu108 create a hydrophobic barrier, blocking the pore (Törnroth-Horsefield et al., 2006) **(Figure 7)**.

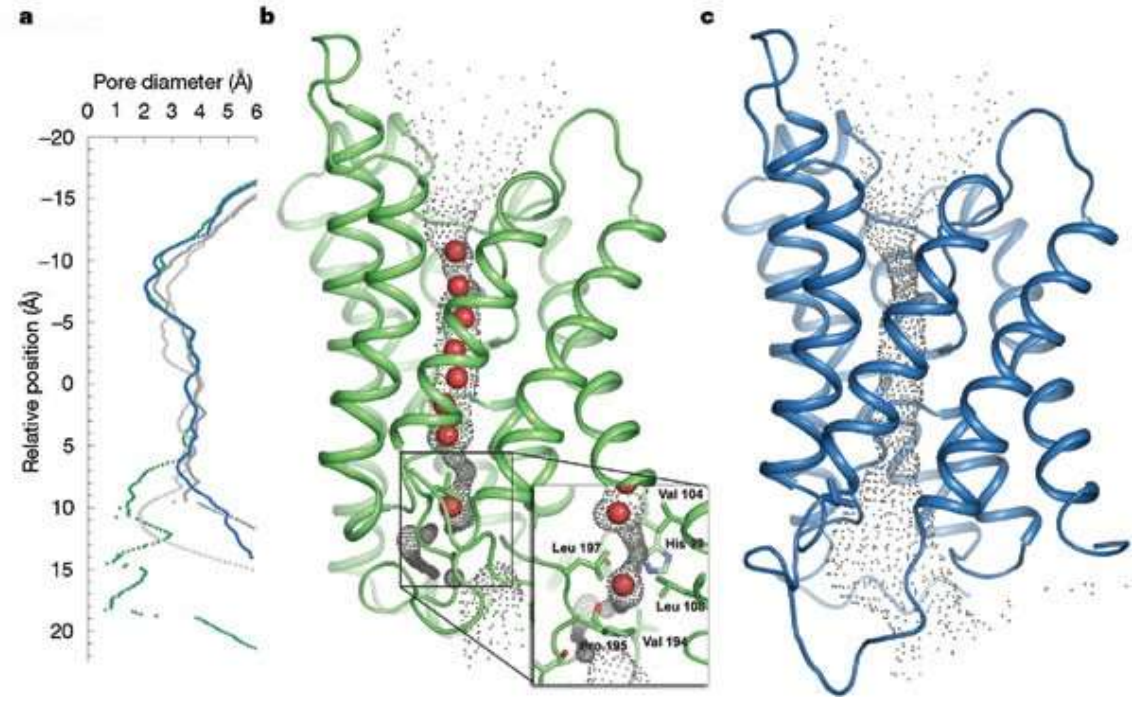

Figure 7. Modeling of SoPIP2;1 **a)** Pore diameter of the open conformation of SoPIP2;1 (blue), AQP0 open conformation (light grey), and AQP1 (grey). **b)** Illustration of the pore with the insertion of the loop D. **c)** Open conformation of SoPIP2;1. Figure from Törnroth-Horsefield et al., 2006.

1.3.3. Heterotetramers and posttranslational modifications

When aquaporins go to the plasma membrane, they are grouped in the form of tetramers. In this structure, each aquaporin monomer acts as an independent channel, but a fifth channel is created due to the central pore, which has proved its ability to transport some solutes like ammonia (Bertl and Kaldenhoff, 2007; Gonen and Walz, 2006).

Due to their similar structure, different aquaporins from the same subfamily can interact between them and assemble together. This plasticity allows different combinations to increase the complexity and the possibilities of transport of different solutes in the same structure (Luu and Maurel, 2013; Yaneff et al., 2014). The heterotetramerization of PIP1s and PIP2s could be related to an ancient ability to guide PIP1s to the plasma membrane, since most PIP1s are retained in the ER

while they are co-expressed with some PIP2, leading to a synergistic effect on water membrane permeability when they are co-expressed in oocytes, increasing the P_f, although not when they are expressed in yeast (Bienert et al., 2018). Furthermore, it has also been discovered that certain residues of each monomer can interact with each other to generate a stable tetramer, like some cysteines (Bienert et al., 2012).

Depending on the sequences of monomers that form the tetramer, the aquaporins will have one level of activity or another. For instance, five isoforms of HvPIP1 co-expressed with HvPIP2;8 inhibited its ionic conductance relative to its activity when not co-expressed. However, when co-expressed with HvPIP1;3 and HvPIP1;4, HvPIP2;8 they maintained the activity transport of Na and K at lower levels, suggesting that heterotetramerization could also serve to modulate the activity of some aquaporins (Tran et al., 2020).

Furthermore, PIPs heterotetramers activity is modulated by some posttranslational modifications such as the intracellular pH of the cell, calcium (Ca) concentration (or other divalent cations) and phosphorylation in conserved residues (Alleva et al., 2006; Johansson et al., 1998; Törnroth-Horsefield et al., 2006; Tournaire-Roux et al., 2003).

A very usual modification that aquaporins could suffer is the protonation of some conserved residues due to cytoplasmic pH during anoxia, closing PIPs channels, or the reduction of the hydraulic conductivity (Tournaire-Roux et al., 2003). In SoPIP2;1, it is known that at low pH, His193 is protonated, leading to a rotation in that chain, preventing to form of a salt bridge to Asp28, affecting the folding of loop D (Törnroth-Horsefield et al., 2006). Another recent study on *Beta vulgaris* L. points out that the pH of the medium will vary the positive charge of the C-terminal region of PIPs, altering the loop D conformation (Scochera et al., 2022).

Cytosolic Ca and other divalent cations have shown that they can regulate transport activity and expression of aquaporins, acting as a secondary messenger in cell signalling (Cabañero et al., 2006; Johansson et al., 1996; Maathuis et al., 2003). In that sense, some articles point out that Ca is involved in regulating aquaporin activity, which inhibited water channel activity when it was combined with $HgCl_2$

(Ionenko et al., 2006). On the other hand, *in vivo* studies have shown that Ca upregulates the activity of some aquaporins like PaPIP1;4 (Breia et al., 2020), or modulates the expression of several root aquaporins under nutrient stresses (Maathuis et al., 2003) or salinity stresses (Martínez-Ballesta et al., 2008).

Furthermore, Ca is related to the state of phosphorylation. That was the case of PM28A which depends directly on submicromolar concentrations of Ca, where the phosphorylation activates the channel (Johansson et al., 1996). In the same way, some aquaporins have shown close conformation when some conserved serine residues are dephosphorylated under drought stress conditions (Törnroth-Horsefield et al., 2006).

1.3.4. Aquaporins in melon

As it has already been seen, aquaporins form a large and complex family of proteins with numerous functions, and melon aquaporins are not an exception. In the first articles, aquaporins in watermelons started to be named "Tetracycline transporter-like protein", but they began to be known as transmembrane protein pools (Rhodes and Zhang, 1995). Historically, aquaporins' activity was measured, even if aquaporins were not well known, through physical parameters of the plants like the transpiration rate, the osmotic potential differences between the soil solution and the root solution, and the root hydraulic conductivity (Adler et al., 1996).

Some of the first articles that talk about aquaporins in melon come from our group. In the first years, the aquaporin activity was measured through the hydraulic conductance (L_o) and they proved the positive effect of $CaCl_2$ for ameliorating the negative effect of NaCl (simulating saline stress), which decreases the L_o (Carvajal et al., 2000). This former article, combined with others, proves that the negative effect of NaCl on water channel activity was not only due to a high ion concentration effect (Martínez-Ballesta et al., 2000). Since that moment, our group has worked with aquaporins in melon until the research performed in this thesis where we have described aquaporins in different conditions and their implication in a physiopathy.

In the last three years, aquaporins in other species related to melon have been described: cucumber and watermelon. For cucumber (*C. sativus* L.), 39 putative aquaporin genes were identified, divided into 19 PIPs, 8 TIPs, 9 NIPs, 2 SIPs, and 1 XIP (Zhu et al., 2019) and for watermelon (*C. lanatus*), 35 aquaporins genes were described, with 16 PIPs, 8 TIPs, 8 NIPs, 2 SIPs, and 1 XIP (Zhou et al., 2019). In this thesis, we have identified the aquaporins in melons and their implication in abiotic stresses and cracking, a very usual physiopathy in melons in our region.

1.4. Abiotic stresses

An environment or ecosystem is made up of different variables that can generate stress for plants. These stresses can be biotic if they are produced by biological entities such as viruses, bacteria, fungi, human action, or abiotic if the source of the stress is not biological. Some of the most important abiotic stresses are salinity, solar radiation, wind, drought or excess water, high or low temperatures, and deficiency or toxicity of some elements, among others (Barzana et al., 2021; López-Ortega et al., 2016).

The plants, due to their inability to move to a place with better conditions, have developed numerous techniques to adapt to abiotic stresses. These adaptations are usually associated with a series of responses at all levels, physiological and genetic, which increase the chances of survival for the plant (Kumar et al., 2020). For their part, aquaporins also mediate numerous processes of adaptation to these stresses, regulating the flow of water inside the cells, the opening or closing of the stomata, or the transport of certain nutrients and stress-signalling molecules such as H_2O_2 (Zhou et al., 2019).

In semi-arid zones in the Mediterranean area, like the Region of Murcia, it is quite common to find some abiotic stresses, standing out salinity, nutrient deficit, and high temperatures stresses (López-Ortega et al., 2016). For this reason, we will now focus on delving into these, since they may also be involved in a very usual physiopathy that affects melon fruit, the cracking.

1.4.1. Salinity stress

Soil salinity is one of the most important environmental factors that decreases plant survival and the productivity of crops in different areas of the world. It affects up to 7% of the world's total land area and about one-third of irrigated land (Flowers and Colmer, 2008; Yamaguchi and Blumwald, 2005). In the same way, salt stress is the main abiotic stress that affects crops around the world, altering the absorption of water and nutrients by the plant (Parihar et al., 2015).

Salinity could be caused by low-quality irrigation water or salinized soils. High concentrations of salts in the growth medium decrease the osmotic potential of the soil, altering the water balance of the plant and the distribution of ions at the cellular and general level of the entire plant (Martínez-Ballesta et al., 2006). What is more, soil salinity affects the growth of plants producing specific toxicities of the ions and an imbalance in the absorption of the rest of the nutrients (Sheldon et al., 2017).

Salinity in soils can be caused by different elements and compounds in toxic concentrations, but the main one responsible for this stress is NaCl. In plants, high cytosolic levels of Na^+ may disrupt K^+ homeostasis because Na^+ is similar to K^+ and many K^+ transporters do not distinguish between these cations. These low levels of K^+ can affect the normal functioning of a large group of enzymes. Moreover, excess external Na^+ can not only impair K^+ acquisition but also leads to the accumulation of Na^+ in plant cells, being toxic to cells (Kronzucker and Britto, 2011; Pardo and Quintero, 2002).

The negative effect of NaCl salinity on plants is generally attributed to the toxicity of Na^+, but chlorine anion (Cl^-) can produce the same effect. Cl^- induces chlorosis and necrosis due to chlorophyll degradation by means of the accumulation of Cl^- in the chloroplast inhibiting photosynthesis through damage in PSII reaction centres (Slabu et al., 2009), and the reduction of function on thylakoid because of the loss of the ion homeostatic control (Herdean et al., 2016). Besides, Cl^- can

compete for the cellular uptake of other anions like nitrate or phosphate producing N or phosphorus (P) deficiency (Li et al., 2017).

At physiological levels, the response of plants to salinity is divided into two steps. Firstly, the independent response of the ion, caused by the osmotic changes, in a matter of minutes or days, produces a stomatal closure and the inhibition of foliar expansion, through Ca and ROS signalling (Gilroy et al., 2014). Secondly, a later response appears, after days-weeks of the exposition, depending on whether the ion concentrations are toxic for the plant, which will accumulate mainly in the old leaves and will produce senescence or even the death of the plant (Negrão et al., 2017).

Plants have three main mechanisms to combat salinity stress. The main one is the exclusion of ions, preventing their uptake. Another one is the compartmentalization of toxic ions in specific tissues, cells, or organelles, moving them away from the most sensitive points. Finally, the last one is the maintenance of the uptake of water regardless of Na^+ levels (Munns and Tester, 2008). Apart from these, the plant regulates itself at a physiological level to maintain its water status by modifying its transpiration or stomatal density (Barbieri et al., 2012; This et al., 2010) or producing antioxidants (Ashraf, 2009).

With high levels of NaCl, plants reduce root L_o. Due to this and to maintain the osmotic adjustment and recover the water balance, the plants need to use some of their energy, negatively affecting their growth rate (López-Berenguer et al., 2006). Aquaporins are responsible for root L_o so it is not surprising that they are widely involved in the adaptation of plants against saline stresses, changing their expression and content in their target membrane (Aroca et al., 2012). In fact, it is known that a NaCl treatment reduces the expression in most aquaporins during the first 2-5h, with a fast decline of water status in plants. After this, some articles have reported an increase in expression (Maathuis et al., 2003), but most of them revealed a widespread decrease in long-term salinity stress, showing some differences between species and the length of treatments (Yepes-Molina et al., 2020).

The most researched way in which plants act on aquaporins is by regulating their transcript. A decrease in the transcription usually means a decrease in the amount of protein generated from this aquaporin, mainly to decrease the water transport into the cell against stresses (Kapilan et al., 2018; Martínez-Ballesta et al., 2006). That is the case of *A. thaliana* L. which reduces the abundance of PIP and TIP transcripts long-term after salt treatments (Afzal et al., 2016; Kapilan et al., 2018; Muries et al., 2011; Sutka et al., 2011).

Although this generality of reduced expression tends to be fulfilled in numerous species and aquaporins, there are cases in which we can find increases in expression in specific aquaporins. In the early stages of development, *A. thaliana* L. seedlings have upregulated *PIP2;2* and *PIP2;3* in the aerial part, and *PIP1;1*, *PIP1;2*, *PIP1;3* and *PIP2;7* in roots, (Jang et al., 2004), showing that the plant, despite external saline stress, could be trying to maintain some water transport for seedling development.

On the other hand, some aquaporins like McTIP1;2 in *Mesembryanthemum crystallinum* L. change their positioning, internalizing from tonoplast to endosomal compartments (Vera-Estrella et al., 2004) or AtTIP1;1 in *A. thaliana* L. which goes from tonoplast to intravacuolar invaginations (Boursiac et al., 2005), when they are exposed to saline stresses, contributing to homeostatic maintenance. This also occurs with some PIPs, which have shown internalization through clathrin vesicles or which are associated with rafts domains (Dhonukshe et al., 2007; Li et al., 2011).

In salinity conditions, the aquaporin trafficking is increasing in both senses, endo, and exocytosis from the plasma membrane (Luu et al., 2012) and modifying their phosphorylation status through H_2O_2 signalling (Prak et al., 2008). Furthermore, this abiotic stress induces a higher internalization of GFP-AtPIP2;1 and GFP-AtPIP2;1S283A that mimics the phosphorylated state, compared with GFP-AtPIP2;1S283E in *A. thaliana* L. plants, suggesting that internalization of AtPIP2;1 under NaCl stress requires the non-phosphorylated form of Ser283 (Prak et al., 2008).

1.4.2. Micronutrient deficiency stress

Micronutrients are a group of elements that are essential for the proper functioning and development of plants but are needed in smaller amounts and are found in a lower proportion within the plant than macronutrients. Among the most important micronutrients for most plants, we find B, cobalt (Co), Cu, iron (Fe), manganese (Mn), molybdenum (Mo), Ni, and zinc (Zn) (Shukla et al., 2018).

The availability of some micronutrients like B, Cu, Fe, Mn, Mo, or Zn is not often related to the quantity. These essential micronutrients are subjected to different soil properties such as pH, water status, temperature, organic matter content, redox potential, microorganisms, or other nutrient interactions (He et al., 2005; Mikula et al., 2020). Furthermore, soil degradation as a result of intense cultivation without suitable replenishment of nutrients, poor crop rotations, and the decrease in the addition of organic matter, have led to a reduction in soil quality and micronutrient deficiency, having a negative effect on animal and human health, but also on crop productivity (Shukla et al., 2018).

In plants, depending on which microelement is missing, there will be some symptoms or others, although in most cases there will be a decrease in growth and biomass production as in any other stress for the affection of metabolic pathways, hormonal production, structural deficiencies or decrease in the physiological process like photosynthesis, with visible effects like chlorosis or underdeveloped crops (Mikula et al., 2020).

B is a key element for the formation of plant cell walls, and it is involved in other processes such as hormonal balance, flowering and fruiting in plants, the generation of nucleic acids by incorporating P into their chain (Macho-Rivero et al., 2017) and plant resistance to low temperatures (Porcel et al., 2018). Its deficiency generates disturbances in the growth of plants, with smaller and cracked fruits and yellowish and brittle leaves, which implies a further significant reduction in quality and yield (Camacho-Cristóbal et al., 2008; Shorrocks, 1997).

Cu plays an important role in cell wall metabolism, signalling to the transcription protein trafficking apparatus and oxidative phosphorylation. Furthermore, Cu is an important component of many enzymes and is involved in resistance against fungi and bacteria (Yruela, 2009). On the other hand, its deficiency generates in the plant a whitening of the leaves along with in addition to a slowdown of the maturation processes (Kumar et al., 2021).

A very typical micronutrient deficiency is Fe, although Fe is a very abundant element in the soil, it is normally presented as insoluble forms like Fe^{3+}. To improve absorption, the plants themselves can reduce this iron to Fe^{+2} or use natural chelators (Briat et al., 2015). Fe is involved in the transfer of electrons, and it is also essential in the synthesis of chlorophylls. The main deficiency symptoms associated are chlorosis in leaves and changes in the colour of the plant from green to yellow-white due to loss of chlorophyll (Kobayashi et al., 2019).

Mn is an important cofactor mainly involved in respiration processes as well as serving as a link and as a bridge between numerous substrates and proteins. Its deficiency generates browning and the fall of the leaves, after incipient chlorosis (Millaleo et al., 2010).

Mo stands out in its role as a N fixer due to its oxide-reducing properties, in addition to being an important element against other stresses. Its deficit generates a reduction in chlorophyll levels (Kaiser et al., 2005).

Zn participates in the metabolism of certain molecules such as carbohydrates and proteins, is a cofactor for numerous enzymes and participates in some of their catalytic properties, and is also involved in gene transcription. Its deficit affects gene transcription, auxin synthesis and generates chlorosis (Brown et al., 1993).

Since some aquaporins are able to transport some micronutrients like B (Pommerrenig et al., 2015) and the transport of other micronutrients is associated with the passive transport through water flow (He et al., 2005), aquaporins could be related to some micronutrient deficiencies.

The main microelement related to aquaporins is B, which is transported mainly by NIPs. AtNIP5;1 has shown boric acid transport activity in oocytes. In plants, this aquaporin expression is upregulated under B deficiency. Also, when *A. thaliana* L. mutants for NIP5;1 are grown under B deficiency, their growth is inhibited (Takano et al., 2006). Another aquaporin, OsNIP3;1 is found to be a B channel in rice (Hanaoka and Fujiwara, 2007). Other studies point to the possible implication of other aquaporins in B transport like HvNIP2;1 in barley (Schnurbusch et al., 2010), and even some aquaporins with other subfamilies like AtTIP5;1 (Pang et al., 2010) or OsPIP1;3, OsPIP2;4, OsPIP2;6, and OsPIP2;7 (Kumar et al., 2014; Mosa et al., 2016).

Apart from B, other relations between microelements and aquaporins regulations have been observed. Ca starvation in *A. thaliana* L. has shown a significant decrease in the transcript levels of many isoforms in all subfamilies (Maathuis et al., 2003). In the same line, deficiencies in Fe decreased the amount of protein of some aquaporins like ZmPIP2;2 in *Z. mays* L. (Hopff et al., 2013). Other studies associate this same aquaporin decrease in Arabidopsis plants under H_2O_2 treatments (Hooijmaijers et al., 2012), linking this stress signal to deficiencies and pointing to an important molecule that regulates the expression of aquaporins.

For other microelements like Zn, there are some controversies. In the same line, it is known that in deficiency conditions, several PIPs decrease their expression in *H. vulgare* L. (Gitto and Fricke, 2018). Notwithstanding, increases in Zn can also inhibit (Yukutake et al., 2009), upregulate at transcriptional and post-translational levels (Ariani et al., 2019), or increase the activity of some aquaporins (Németh-Cahalan et al., 2007).

Si is not considered a micronutrient, although sometimes it is known as a "quasi-essential element" (Seal et al., 2018). However, due to the importance it has had in relation to this thesis, a comment about it will be included. Si is an element that improves adaptation to biotic abiotic stresses in plants (Sivanesan et al., 2014). It is well known that the presence of this element could prevent damage in the plants and stabilize the membrane lipids (Agarie, 1998; Seal et al., 2018).

Furthermore, the Si accumulation is related to some abiotic stresses like P deficit or high temperature alleviating their negative effects (Zhang et al., 2019). Si in the form of silicic acid, $Si(OH)_4$, enters the plant roots mainly by water flow via apoplastic and symplastic pathways, involving this last some aquaporins (Jian et al., 2006). Even, some Si transporters, like Lsi1 (OsNIP2;1) or Lsi6 (OsNIP2;2), are aquaporins, NIPs-III type (Chiba et al., 2009; Yamaji et al., 2008).

On the other hand, in pumpkin (*C. moschata* L.), some changes in the residue in position 242, a proline to a leucine, have been described and they could be involved in Si absorption. This genetic variation is the most preserved characteristic in NIPs-III, which are aquaporins with Si transport ability (Mitani et al., 2011). As regards the NPA motif in Si aquaporin transporters, they change it most of the time into NPV motifs as can be seen in Cucurbitaceae species like *C. melo* L. and *C. sativus* L., which have NPV motif in their loop E (Deshmukh et al., 2020). Therefore, studying melon sequences to analyse the implication of these changes in their activity may be interesting in order to understand the Si transport in melons.

1.4.3. High temperature stress

Due to climate changes high temperature is one of the main environmental factors that hinders plant growth and that affects agricultural production, such as the yield reduction, decrease in fertile lands, or optimal water use (Lohani et al., 2019). High-temperature stress is defined as a series of irreversible damages to the metabolism, development, and structures of the plant due to excessive heat. Currently, with the rise in the average temperature of the Earth due to climate change, this stress is becoming highly relevant due to the loss of cultivable areas (Prasad et al., 2017).

This abiotic stress depends on three parameters in turn, which will indicate the degree of intensity: the duration of the stress, the rate of increase in temperature, and the maximum temperature. Each plant species has a minimum and maximum survival temperature and an optimal temperature for its correct development, which

for almost all species is below 50 ºC since from this temperature severe damage appears (Porch and Hall, 2013).

The stress induced by high temperatures in plants can generate direct changes such as physiological ones (respiration, membrane stability, or alterations in development) or indirect changes derived from the former such as modifications in the evaporative demand, the energy balance of the leaves, or changes in stomatal behaviour (Wahid, 2007).

Exposure to high temperatures triggers a cascade of genetic signalling and activation that seeks to stabilize proteins and membranes and the production of antioxidant enzymes that protect against oxidative stress caused by high temperatures (Almeselmani et al., 2006; Niu and Xiang, 2018). Further, heat stress is especially key in processes involved in male and female reproductive development, such as morpho-biochemical variation in the pollen and stigmatic tissues, pollen ROS production, seed number or weight, and abortion (Aiqing et al., 2018; Bheemanahalli et al., 2019; Prasad et al., 2017).

To avoid these damages, plants employ numerous mechanisms like scavenging of ROS, production of antioxidants, accumulation of osmolytes, and synthesis of chaperones such as heat-shock proteins (HSPs) and late embryogenesis abundant (LEA) proteins (Kotak et al., 2007). Some of these genetic changes modify the aquaporin expression patterns and water relations since temperature stress affects the root L_o, which is directly related to aquaporins (Aroca et al., 2005). It is known that in high-temperature stress, plants modify their aquaporin expression to adapt to this stress.

Although it is generalized as a decrease in expression after long-term stress, different plants adapt differently to different intensities and times of stress. For example, in soybean, in roots, PIP1;7, PIP1;8, PIP2;4, PIP2;5, PIP2;13, PIP2;14, TIP1;7, TIP2;2, and TIP2;6 are transcriptionally upregulated during the first six hours after heat treatment, whereas TIP4;1 and SIP1;3 were downregulated. In leaf, all were downregulated after 12 hours, but with 6-hour treatment, PIP1;7, PIP1;8, PIP2;4, PIP2;13, PIP2;14, TIP2;2, TIP2;6, and SIP1;3 were upregulated (Feng et al.,

2019). Some plants like *Rhazya stricta* L. have shown an adaptation based on increased PIP1;2 and PIP2;1 aquaporins expression in leaves at the hottest daylight hours (Obaid et al., 2016), or strawberry (*Fragraria vesca* L.) that increases PIP expression after one-hour heat stress (42 ºC) (Christou et al., 2014). Meanwhile, in other plants like tobacco (*Nicotiana tabacum* L.), there is a decrease in the PIP2s levels in roots after 50 ºC treatment (Hamachi et al., 2019) or tomato, in which some aquaporins seem not to be affected by high temperatures (Giri et al., 2017).

Aquaporins are also able to present different patterns of expression between cultivars in response to heat stress as happens in *Setaria italic* L., where PIP1;2, PIP3;1, SIP1;1, NIP1;2, and TIP2;2 were increased but unevenly (Singh et al., 2019). Due to this enormous variability, it is interesting to study this stress to identify patterns that help us improve treatments against that stress.

Until now, it is unknown how aquaporins are affected by high-temperature stress in melons. However, recently, it has been shown that a foliar application of putrescine before short-term heat stress improves the quality of fruits and their tolerance to this abiotic stresses, increasing total sugars, polyamines content, antioxidant capacity, and mineral content (Piñero et al., 2021). Therefore, the melon seems to resist high-temperature stress well and it could even improve its organoleptic characteristics.

1.5. Cracking physiopathy

Cracking or splitting is a physiopathy that consists of a mechanical break or fracture in the fruit cover, making it unusable for consumption and producing numerous economic losses (Peet, 1992). This physiopathy affects numerous types of crops, highlighting the tomato (Matas et al., 2004), the jujube (Ren et al., 2017), the pomegranate (Dichala et al., 2018), the apple (Joshi et al., 2018), the citrus (Li and Chen, 2017), or the melon (Qi et al., 2015) among others, so it is a very widespread problem.

This fracture can be due to both external and internal causes. Among the most important external causes are those related to the climate (intense rains, sudden changes in temperature, air humidity, and solar radiation), soil conditions (such as water retention or dissolved nutrients), fertilizers (composition and chemicals), and the handling of the fruits once harvested (Cline et al., 1995; Fernández-Trujillo et al., 2013; Khadivi-Khub, 2015; Li and Chen, 2017).

On the other hand, there are a series of internal components that can also produce this physiopathy, such as the imbalance between internal and external pressure, the mineral composition of the fruit, and its water content (Cline and Trought, 2007; Joshi et al., 2018; Schumann et al., 2014; Winkler and Knoche, 2019). Furthermore, in terms of the physiological components, some evidence indicate that this disorder has a genetic component, since some cultivars are more susceptible than others, increasing the severity and the incidence of this physiopathy (Fernández-Trujillo et al., 2013; Joshi et al., 2018).

The evident relationship of water with this physiopathy, like the imbalance between the internal pressure and external growth (accumulation of water that increases faster than the growth of the rind) or the importance of aquaporins regulating water and solutes transport and other physiopathies (Barzana et al., 2021; Cabañero and Carvajal, 2007; Hagassou et al., 2019; Shen et al., 2014), leads us to think about the possible implication that aquaporins may have in the cracking physiopathy.

In that line, some recent studies start signalling the implication of aquaporins in cracking. For example, in jujube, an RNAseq analysis signals PIPs aquaporins, tubulin, calreticulin, and calmodulin as probably genes involved in jujube cracking (Ren et al., 2017). On the other hand, some resistance genes to cracking are already known in apples, and some aquaporins were found to correlate an increase in expression with a reduction in the number of cracked fruits (Joshi et al., 2018).

What is more, other authors described that the aquaporin PaPIP1;4 in *Prunus avium* L. (sweet cherry) was upregulated after a Ca treatment to decrease the cracking (Breia et al., 2020). However, it has been described that some aquaporins

in sweet cherries decrease their expression towards maturity (Chen et al., 2019), pointing out that maturity is not the main reason that causes the cracking and the changes in water relation flow through aquaporins. As a result, there is controversy about the way in which aquaporins could be intervening in the maturation process and in the appearance of this physiopathy.

In melons, the implication of aquaporins in the cracking is not well described yet, but some authors have studied some important relations between the cracking in melons and the water. Nevertheless, for several years, evaluation models and methods have started to standardize to detect the genes responsible for the appearance of cracking and resistance genes against them, also finding the most sensitive and resistant varieties (Qi et al., 2015).

1.5.1. Cracking in melon

Among the main causes of melon cracking are the differences in temperature between day and night, high levels of sugars in the pulp of the fruit during ripening, extreme levels of soil moisture during ripening, or the reduced space between the melon plants (Fernández-Trujillo et al., 2013) **(Figure 8)**.

Figure 8. Model melon “Piel de Sapo” selected for carrying out the tests of this thesis in which cracked melons were involved. Own image.

Melon cracking does not affect all varieties equally. Some types of melons with thinner skin, like *C. melo Conomon*, or with higher soluble solids (11% to 15%) and a sweeter flavor tend to suffer a higher incidence, such as Hami-type melons (*C. melo Reticulatus*), Charentais (*C. melo Cantalupensis)*, Canarias (*C. melo Inodorus*) or "Piel de Sapo" (*C. melo Inodorus*), which is the most common cultivated in Spain (Fernández-Trujillo et al., 2007). For this reason, this variety was selected to perform this thesis.

As regards other possibles causes to take into consideration during the ripening process until maturity, melons modify the enzyme activity, discompose their cell walls, accumulate sucrose in mesocarp cells, which can act as an osmolyte, and increase the ethylene levels (Kyriacou et al., 2018). All these changes are related to cracking incidence, increasing the probability of the appearance of this physiopathy, which means that excessive melon ripening will lead to greater economic losses.

Taking into account the area in which we find the main stresses described above and their possible involvement in the development of melon cracking, we can find some points that could be acting as a link between abiotic stresses and melon cracking **(Figure 9)**.

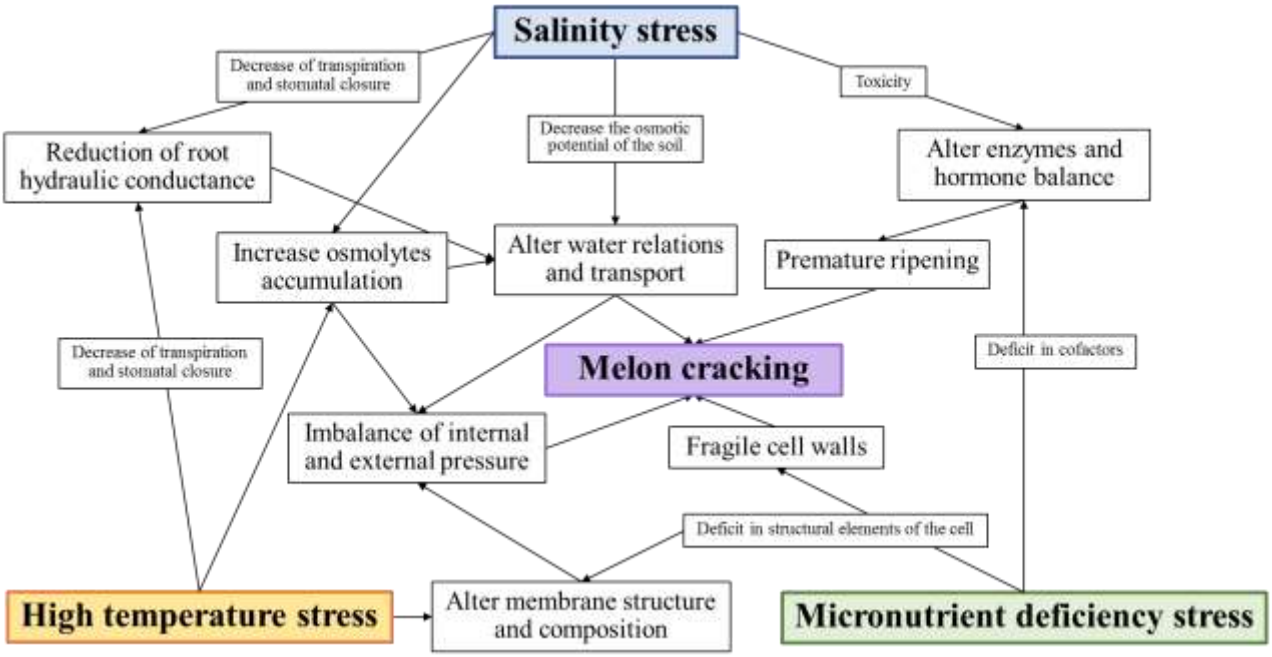

Figure 9. Possible relations between the abiotic stresses studied and the cracking in melon plants. Own figure.

1.5.2. Foliar treatments against cracking

Foliar treatments are composed of elements that can be absorbed directly via leaf stomata and hydrophilic pores in the leaf cuticle and go to the different parts of the plants (Fernández and Brown, 2013). This nutrition allows a faster response of the plant than soil fertilization, improving the time reaction of the plant to the treatment and getting better (Niu et al., 2021).

Regarding the cracking disease, some foliar treatments have proved to decrease the cracking incidence. However, we can find a plethora of differences between treatments and species. One of the most widespread treatments to reduce cracking is Ca. This treatment has been used in litchi (Dinesh et al., 2017), pomegranate (Davarpanah et al., 2018), lemon (Bakshi, 2018), blueberry (Marshall et al., 2019), and sweet cherry (Breia et al., 2020).

In that line of knowledge, Ca could be decreasing the aquaporin activity and their permeability or other ions (Tran et al., 2020), decreasing the water uptake or controlling the balance of nutrients. In addition, Ca has been proven to decrease the

respiration rate and the production of ethylene, delaying the ripening process (Koutinas et al., 2010; Vangdal et al., 2008).

Possibly the second most used elements to combat cracking in different fruits are B and Zn (Chater and Garner, 2018; Ihsan ul and Abdur, 2012). B is a micronutrient that could be contributing at a structural level so that the plant has enough B to continue building its cell walls and thus allow better growth of the fruit as well as at a signalling level (O'Neill et al., 2004), in addition to acting synergistically with Ca, maintaining the pectin structure in plants (Shi et al., 2017).

In turn, Zn could be acting as an inhibitor of the activity of some aquaporins, as has already been shown on other occasions, reducing water uptake and, with it, the internal pressure that the fruit may be suffering (Németh-Cahalan et al., 2007; Yukutake et al., 2009), which is also involved in the correct metabolism and transcription of the plant (Brown et al., 1993).

1.5.3. Foliar treatments in melon

Mineral foliar treatments are widespread in melon plants to increase the production of the plants and the quality of the fruits. For example, it is known that melon plants tolerate high levels of B, decreasing the water transport and the general transport of nutrients (Goldberg et al., 2003), while very high levels, more than 10.4 mg L^{-1}, could decrease the production (Edelstein et al., 2007).

Also, B in combination with Zn increased melon leaf area, the number of female flowers, and total yield (Sabri et al., 2021). Furthermore, the application of zinc sulfate increased the leaf chlorophyll, the dry weight of leaves, and the yield of melon plants (Nasiri Dehsorkhi et al., 2020). Additionally, zinc oxide particles have been shown to decrease cadmium (Cd) phytotoxicity (Shah et al., 2021).

On the other hand, Zn also proved to increase the yield and nutraceutical content, like the flavonoids. Furthermore, it could be an option to combat the malnutrition of this micronutrient since an accumulation of Zn in its pulp could be

proved (Rivera-Gutiérrez et al., 2021) even when melon has not a very good absorption capability with respect to Zn (Yılmaz and Tugrul, 2022).

Other elements like Ca have shown an increase in the fruit firmness, directly related to the rind and possibly to the cracking, and it has improved the storage life in muskmelon (Johnstone et al., 2008). Moreover, $CaCl_2$ has been shown to decrease the negative effect of saline stress, decreasing the L_o (Carvajal et al., 2000), which could be involved in cracking incidence.

In another study, Si has shown to increase the weight, number, and tonnage of melon fruits, further increasing the nutrient uptake of N, P, K, Ca, Mg, Fe, Mn, and Zn, but it did not change the pulp firmness or soluble solids (do Nascimento et al., 2020). Previous studies in melon show how Si application increased assimilation rates of nitrates and boosted shoot biomass production getting a better crop performance and lower environmental impact (Neocleous, 2015). Further, other studies on melon already proved they are beneficial to other pathologies like powdery mildew, reducing the severity of the disease, and increasing the water content in leaves (Buttaro et al., 2009), so it could be acting through aquaporins.

That is why, combining the most commonly used elements for the treatment of cracking in other plants, with the treatments that are most used to improve the qualities of the melon and the elements that could be more related to the aquaporins, it was decided to carry out the treatments foliar of this thesis to prevent the appearance of cracking with Ca, B, and Zn. Si was omitted from these treatments for not presenting deficiency problems in the soils of this area.

Justification and objectives

2. Justification and objectives

The melon is one of the most produced fruits in Spain, especially in our south-eastern region, even though the fact that it is an area with frequent abiotic stresses such as salinity, high temperatures, and nutrient deficiencies. Given the importance of aquaporins in the adaptation of plants to abiotic stresses, it makes them essential to understanding the biological changes in plants under these conditions, being this knowledge necessary to increase crop production and avoid these stresses or improve the plant's response to them.

On the other hand, in our first hypothesis, we postulate that cracking, a physiopathy that consists of the appearance of cracks in the fruit's rind, could be due to an excessive intake of water by the plant and the fruit, increasing its internal pressure. In such a way, an imbalance in the aquaporins was placed as a possible cause of the appearance of this physiopathy.

For all these reasons, in the first part, we had to identify all the aquaporins present in the melon and lay the foundations on which we could continue working. Thus, the realization of this research work is justified by the following aspects:

I. Before this thesis, the aquaporins of *C. melo* L. were not well defined and named based on basic concepts such as similarity with other proteins of other model species such as *A. thaliana* L., *O. sativa* L., or *Z. maize* L., or other phylogenetically close species such as *C. sativus* L. or *C. lanatus* L.

II. It is necessary to deepen the knowledge about aquaporins and abiotic stresses relations, especially in melon, since it is usually subjected to abiotic stresses due to the locations where it is usually grown.

III. Due to its significant economic impact, it is essential to improve its production and yield, preventing the appearance of physiopathies such as cracking, which prevents the fruit from being marketed. The development of technologies or treatments to prevent and reduce the incidence of cracking is essential in this regard.

IV. Studying aquaporins and their involvement in cracking opens a promising door as regards the reduction of the appearance of this physiopathy by regulating aquaporins, but the scant bibliography in this regard means that more studies are required in this regard to broaden knowledge in this field.

2.1. Main objective

Taking these premises into consideration, this Doctoral Thesis has as its main objective to describe all melon aquaporins and their involvement in the most important abiotic stresses in our region (salinity, high temperature, and micronutrient deficiency) and a very common physiopathy, the melon cracking, which consists of an imbalance between the internal and external pressure of the fruit, which generates cracks in the rind of the fruit, impeding its commercialization.

2.2. Specific objectives

This main objective has been developed through the following specific objectives:

I. To characterize the existing aquaporins in melon, establishing their name based on phylogenetic studies and comparing their sequences with those of aquaporins from other species, analyzing the conserved residues to study the possible molecules that they are capable of transporting, and studying the expression profile of all aquaporins in root and leaf.

II. To research the behaviour of melon plants under abiotic stresses (salinity, high temperature, and micronutrient deficiency) at a physiological level, their nutritional composition, and the expression of the aquaporins in roots and leaves in a culture chamber (controlled conditions).

III. To study the cracking of melon fruit through field experiments, treating plants with Ca, B, and Zn foliar applications to decrease the incidence of the

cracking and acknowledge the relationship between these elements, Si and cracking.

IV. To analyse the aquaporins expression patterns in the melon pulp to determine if there are any that may be involved in the physiopathy and function as a marker. In addition, to test the transport capacity *in vivo* of selected aquaporins for water, B, and Si in *Xenopus laevis* oocytes.

Results: Chapter I

3. Results: Chapter I. Genome-wide analysis of the aquaporin genes in melon (*Cucumis melo* L.)

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Melon (*Cucumis melo* L.) is a very important crop throughout the world and has great economic importance, in part due to its nutritional properties. It prefers well-drained soil with low acidity and has a strong demand for water during fruit set. Therefore, a correct water balance—involving aquaporins—is necessary to maintain the plants in optimal condition. This manuscript describes the identification and comparative analysis of the complete set of aquaporins in melon. 31 aquaporin genes were identified, classified and analysed according to the evolutionary relationship of melon with related plant species. The individual role of each aquaporin in the transport of water, ions and small molecules was discussed. Finally, qPCR revealed that almost all melon aquaporins in roots and leaves were constitutively expressed. However, the high variations in expression among them point to different roles in water and solute transport, providing important features as that CmPIP1;1 is the predominant isoform and CmTIP1;1 is revealed as the most important osmoregulator in the tonoplast under optimal conditions. The results of this work pointing to the physiological importance of each individual aquaporin of melon opening a field of knowledge that deserves to be investigated.

Chapter II

4. Results: Chapter II. Relationships between aquaporins gene expression and nutrient concentrations in melon plants (*Cucumis melo* L.) during typical abiotic stresses

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Melon (Cucumis melo L.), a member of the Cucurbitaceae, in Mediterranean regions is usually affected by abiotic stresses like salinity, nutrients deficiency or high temperature. These abiotic stresses have been shown to produce the modulation of gene expression as a response to the altered conditions. Among these genes, aquaporins (transmembrane proteins) stand out due to their vital function as transporters of water and different solutes. For this reason, the aim of this work was to study the expression levels of all (31) aquaporins of melon plants (CmAQPs) after exposure to salinity (50 mM NaCl), nutrient deficiency (10% Hoagland solution) or high temperature (40 ºC for 1 h/day) and relate them with nutrient content, water relations and hydraulic conductance. There were general decreases in plant nutrient concentrations, especially in the root (Fe, K, Mn or Zn), while the concentrations of some elements for each stress (B, Ca, Mg, Mo or Si) increased. Physiological parameters were regulated depending on the treatment, showing the important role of hydric physiology regulation in the whole melon plant response to the different stresses. For most of the aquaporins, their expression decreased in the root (PIP2;1, PIP2;5 and PIP2;6 within the PIPs; most of the TIPs; NIP6;1, NIP7;1; SIP1;1) in all three treatments, while other aquaporins were over-expressed, such as the PIP1s (high temperature treatment), PIP2;2 (nutrient deficiency and high temperature treatments) and TIP1;1 (salinity and high temperature treatments) and NIP5;1 (nutrients deficiency treatment). The leaf aquaporin expression levels were less affected. This study shows that CmAQPs expression is modified differently in response to distinct abiotic stresses and that this is related to plant water relations and nutrients levels.

Chapter III

5. Results: Chapter III. Aquaporins involvement in the regulation of melon (*Cucumis melo* L.) fruit cracking under different nutrient (Ca, B and Zn) treatments

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Melon cracking is a physiopathy that is associated with both internal and external changes by the alteration of the water balance and nutrient homeostasis in the fruit. Aquaporins are channels for water and other small solutes, and they are potentially involved in the regulation of melon cracking. In this work, we studied the mineral concentration and expression of all aquaporins in non-cracked and cracked melon pulp in control and after the application of foliar mineral treatments (Ca+B+Zn and B+Zn) in field conditions. Also, we measured the mineral transport of some aquaporins to connect it to cracking. The results showed that both treatments could ameliorate the incidence of cracking. Mineral elements determination showed increases in B, Ca, Si and Zn content in non-cracked Ca+B+Zn treated melons. In control conditions, only NIP2;2 and NIP5;1 had a significant increase in expression in cracked melons compared with non-cracked ones pointing to their involvement in cracking. Furthermore, we were able to verify that the high expression of PIP1;1, PIP1;2 and TIP1;1, which are efficient water channels, was involved in the changes observed in cracking incidence. Finally, transport assays in oocytes were performed with selected isoforms, highlighting the water channel activity of NIP2;2 and NIP5;1, the B channel activity of TIP1;3, TIP1;2, NIP1;1 and NIP2;2, and the Si channel activity of NIP2;2. In conclusion, both effective foliar treatments for avoiding cracking point to the PIP1;1, PIP1;2, TIP1;1 and NIP5;1 and NIP2;2 aquaporins as possible molecular markers.

Discussion

6. Discussion

6.1. Characterization of melon aquaporins

The melon is a plant whose production is widespread throughout the world, although it mainly stands out in warm regions such as those that can be found in our country. This plant, whose genome was sequenced in 2012, has been a subject of study in recent years due to the growth of its economic value (Garcia-Mas et al., 2012). Despite knowing their genome, many genes are not well-described in these plants. Such was the case with aquaporins. Some of these proteins have the fundamental function of transporting water into and out of the cells, although, as has already been described throughout the thesis and in other studies, they also transport numerous solutes and are involved in the balance and signalling of plants (Bienert and Chaumont, 2011; Gilliham et al., 2011; Singh et al., 2020). As a result, the study of these genes becomes fundamental if we want to know specifically the response of water inside the melon plants, and their general state.

When we proceeded to identify them, taking into consideration the melon genome and obtaining the raw data from the NCBI database and the Cucurbit genomics database, we found numerous sequences, which corresponded to scaffolds, transcript variants, or fragments of aquaporins themselves. For this reason, it was essential to order this information and correctly define each aquaporin, to standardize the subsequent study.

In the first study (Chapter I), we were able to find numerous similarities in the aquaporin sequences of the melon (*C. melo* L.) with those of the watermelon (*C. lanatus* L.) (Rhodes and Zhang, 1995) and the cucumber (*C. sativus* L.) (Huang et al., 2009) previously described. Furthermore, the most studied sequences of some experimental models *A. thaliana* L., *Z. mays* L., and *O. sativa* L. were added to the comparison too, allowing the creation of a tree that facilitated the nomenclature of melon aquaporins. This generated numerous name changes that were previously proposed. On the other hand, by locating the genes spatially on the chromosomes,

we were able to describe that some of these aquaporins could have arisen from duplication events (Krasileva, 2019).

In general terms, numerous similarities were found in terms of the number of aquaporins and sequences between the organisms that we compared and melon aquaporins, mainly between PIPs, TIPs, and NIPs, such as the NPA motif. However, we found some differences that stood out such as the absence of NPA motifs in some NIPs (*CmNIP2;1, CmNIP5;1, CmNIP5;2,* and *CmNIP6;1*) SIPs and XIP, where NPA motifs were replaced for the NPV, NPS, NPT, or SPI motifs. Major differences were found in the selectivity filters, both in the ar/R and in the Froger's positions, where only the PIPs had most of the same residues (F, H, T, and R for the ar/R filter and Q, S A, F, and W for the Froger's positions).

Once the residues that could act as filters were identified, the possible functionality of the aquaporins was analysed based on the comparison with orthologous and homologues (Perez Di Giorgio et al., 2014; Soto et al., 2012). That way, the conclusion that the melon PIPs could be mainly involved in the transport of water, CO_2, and H_2O_2 was reached based on previous studies (Anderberg et al., 2012; Azad et al., 2016; Hove and Bhave, 2011). There were also other molecules such as B in the form of boric acid, urea, and As, based on members of the rice plasma membrane intrinsic proteins subfamily which were involved in As permeability and tolerance in plants or permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash (Dordas et al., 2000; Matsumoto et al., 2009; Mosa et al., 2012; Perez Di Giorgio et al., 2014).

Melon TIPs aquaporins were classified into four groups to propose possible molecules transport, attending to the ar/R filter according to other studies (Wallace and Roberts, 2004). Group I is formed by CmTIP1;1, CmTIP1;2 and CmTIP1;3, Group IIa is formed by CmTIP2;1 and CmTIP2;2, Group IIb is constituted by CmTIP3;1, and CmTIP4;1 and Group III only has CmTIP5;1. In general, CmTIPs were predicted to transport nitrogenous compounds (NH_3 and urea) as many other known TIPs (Tyerman et al., 2017), as it has been mainly seen when the H2 and H5 positions have H or I with a non-polar aa (A or G) in LE1 (Jahn et al., 2004).

Furthermore, H_2O_2 transport for group I is possible according to other studies (Azad et al., 2016; Perez Di Giorgio et al., 2014).

NIPs, in addition to being capable of transporting water, glycerol, and other molecules, are known to be capable of transporting metalloids such as B, Si, As, or Sb (Pommerrenig et al., 2015). The eight NIPs found were classified into three groups based on their ar/R filter (Mitani et al., 2008). CmNIP1;1 and CmNIP4;1 belong to Group I, CmNIP5;1, CmNIP5;2, CmNIP6;1 and CmNIP7;1 belong to Group II, and CmNIP2;1 and CmNIP2;2 belong to Group III. Their transport prediction suggests a big range of molecules that could be transported by melon NIPs such as H_2O_2, As, Sb, urea, B, Si, or glycerol (Bienert et al., 2008b).

Regarding the transport of SIPs and XIPs, fewer studies help us to predict their possible transport functions. However, it is known from other species with similar ar/R filter and FPs, that they could be involved in urea transport in the case of SIP1;1, (of *Sorghum bicolour* L. and *Brachypodium distachyon* L.) or H_2O_2 and As for SIP2;1 of the species *Sorghum bicolour* L., *Panicum virgatum* L. *Setaria italica* L. (Azad et al., 2016). Finally, for CmXIP1;1, we did not find aquaporins from other species that would have the same residues in the hot spots that could act as filters. Although there are some aquaporins in *Ricinus communis* L., *Lotus japonicus* L., *Prunus persica* L., and *Glycine max* L., similar to melon, that they show a rare variation in the first NPA motif, this being SPI instead of the usual SPA present in those other species (Lopez et al., 2012) and also it is quite odd to find a mutation in the second NPA motif, being present in *C. melo* L. and other *Cucumis* members SPA instead of the normal NPA sequence.

On the other hand, we were able to verify the aquaporins that had the major expression in leaves and roots in control situations (Chapter I and Chapter II), highlighting the aquaporins CmPIP1;1, CmPIP1;2, and CmTIP1;1. This was repeated when we applied abiotic stresses (Chapter II) where other interesting patterns also appeared in NIPs that could be more associated with the transport of certain elements, and also when we studied the expression levels in the fruit (Chapter III), changing the patterns of expression in this case, increasing the expression of

some aquaporins such as *CmTIP1;3*, *CmTIP2;2*, and *CmXIP1;1*, which appears to be fruit specific, and decreasing others like *CmPIP1;2* or *CmNIP5;1*.

As it has been seen throughout the thesis, aquaporins play a fundamental role in transporting not only water but also different molecules and elements, so it is normal to think that they are involved in the regulation and adaptation of plants to different abiotic stresses, especially those related to water, as well as to different physiopathies that may occur in plants (Barzana et al., 2021; Cabañero et al., 2006; Li et al., 2014; Shen et al., 2014).

6.2. Water transport aquaporins

In our results, it has been clearly shown that PIP, and specially PIP1s, has the main role in avoiding stress and controlling of water supply to the tissues, directly affecting L_o and transpiration parameters in plants. PIP1s are aquaporins that cannot go to the membrane if they are not accompanied by PIP2s and, once there, they regulate them with a synergic effect, increasing the cell P_f (Bienert et al., 2018; Gaspar et al., 2003; López-Pérez et al., 2009; Pawłowicz et al., 2017).

In article 2 (Chapter II), we were able to verify how there were significant increases in the expression of both *CmPIP1;1* and *CmPIP1;2* in the root in the high-temperature treatment. This fact can maintain the levels of water uptake, even though there is a decrease in the expression levels of other PIP2s aquaporins. This can be explained since it is known that in the formation of the tetramer when PIP1s interact with PIP2s, they increase their permeability and water transport (Bienert et al., 2018; Obaid et al., 2016; Otto et al., 2010; Vajpai et al., 2018). These increases of expression in PIP1s could keep or even increase the water uptake through the roots, in response to this increase in temperature as can be seen in other articles (Hussain and Maqsood, 2011).

Furthermore, an increase in the expression of *CmPIP1;1* in salinity stress in leaves could be observed, as it also happens in other articles (Jang et al., 2004), indicating the possible maintenance of the transpiration rate that we could observe,

with no significant changes between our control and salinity treatment which is already described (Aroca et al., 2006; Sade et al., 2009).

In the same line, in article 3 (Chapter III), we could appreciate how cracking was directly associated with an increased expression of the aquaporin *CmPIP1;1* and *CmPIP1;2*, which indicates changes in the water intake towards the fruit, with a consequent and possible imbalance in the internal pressure that could be triggering a cracking principle (Qi et al., 2015). Furthermore, we could observe a decrease in the expression associated in both non-cracked melons with the two treatments proposed based on previous studies (Lopez-Zaplana et al., 2020a) in which we were able to reduce the incidence of cracking.

According to previous research, it seems noticeable that water excess is the main responsible for fruit melon cracking incidence (Fernández-Trujillo et al., 2013; Lopez-Zaplana et al., 2020a), and a quite relevant factor to induce cracking in other species (Cline et al., 1995; Khadivi-Khub, 2015; Li and Chen, 2017; Simon, 2006). PIPs, as fundamental water transporters (Katsuhara and Hanba, 2008), are strongly related to it, and some studies already relate them to cracking (Li et al., 2014).

For the rest of the PIPs (Chapter II), most of them decreased their expression in roots in most of the stresses, protecting plants from external stresses like salinity (López-Berenguer et al., 2006; Zhu et al., 2019) or high-temperature (Martínez-Ballesta et al., 2009). Meanwhile, the decrease in micronutrients could affect the normal activity of the plant by itself (Shukla et al., 2018), decreasing the general aquaporin expression.

Among all the TIPs, CmTIP1;1 was revealed as the most important osmoregulator in the roots, leaves, and fruits of melon plants, both for expression levels in control conditions (Chapter I) and also for its expression changes under salt stress, nutrient deficiency, and high-temperature stresses (Chapter II); in addition to changes due to the situation of cracked/non-cracked melons (Chapter III).

Meanwhile, most of the TIPs decrease their expression in the roots, the same that occurs to PIPs, denoting the prevention of stress damage and the regulation of

the water and nutrients transport (López-Berenguer et al., 2006). However, the aquaporin *CmTIP1;1* was the only TIP that increased its expression levels after subjecting the plants to saline stress and high temperature in their roots. These significant differences signal the importance of CmTIP1;1 in the inherent tolerance of melon plants to such stresses, as it has already been known that this is an important gene that is upregulated under saline conditions (Pawłowicz et al., 2017; Zhu et al., 2019) or high-temperature conditions (Shafqat et al., 2021) in other studies.

On the other hand, and in the same way as it happened with the PIP1s, this aquaporin presented significantly higher values in the cracked melons than in the non-cracked ones. This change in expression could be associated with an increase in water transport to the tonoplast, increasing cell turgor and generating a growth in internal pressure (Leitão et al., 2014) or/and an increase in stress signalling by H_2O_2 (Schüssler et al., 2008) due to cracking or because of it.

Other aquaporins that showed interesting relation with cracking were CmNIP2;2 and CmNIP5;1, as both isoforms were the only ones that were increased in expression in cracked melons in control conditions. Furthermore, we were able to verify through the expression in *Xenopus* oocytes that both CmNIP2;2 and CmNIP5;1 were capable of facilitating the diffusion of water through the plasma membrane. Knowing that other studies are beginning to relate the increased expression of some aquaporins with cracking (Li et al., 2014) and the possible interaction of increased water intake with the appearance of cracking (Chen et al., 2019; Schumann et al., 2014), the relationship between the five mentioned aquaporins and the cracking becomes clear. Finally, CmTIP1;3, CmTIP2;2, and CmNIP1;1 were tested in oocytes, but they did not show any water transport activity.

6.3. Nutrient transport by aquaporins: B and Si.

We also tested the implication of some important aquaporins related to cracking to the transport of B and Si (Chapter III). For TIPs, it was found that the

suggested transport of B (Chapter I) by *CmTIP1;3* was indeed one of its main functions, indicating that its upregulation in leaf tissue under nutrient deficiency stress and high-temperature stresses (Chapter II) could be related to the tolerance of melon plants to this conditions. Indeed, its role in reducing cracking incidence could be observed in the way that control cracked melons have minimum values of expression, but this expression increases with the B+Zn treatment in non-cracked ones pointing to its role in cracking resistance. This hypothesis was confirmed through *in vivo* assays where we could see B transport in *CmTIP1.3* microinjected oocytes (Chapter III).

In previous studies, the application of micronutrients (including B) has proved to reduce fruit cracking (Lopez-Zaplana et al., 2020a), especially under "double irrigation" conditions, which is related to a diminution of nutrients in the soil due to percolation. The relation between this aquaporin and the nutrient deficiency seems to be clear. Indeed, under high "conductivity irrigation", which implies osmotic (salinity) stress, the effect of micronutrients application in the reduction of cracking incidence was not detectable, and in comparison with salinity stress applied in controlled chambers (Chapter II), this aquaporin was not modified under such conditions. These results clearly point to CmTIP1;3 as a fundamental isoform related to stress and physiopathies resistance based on B transport between cytoplasm and vacuoles.

Another interesting TIP was *CmTIP2;2*, which together with *CmTIP1;3*, were the aquaporins that showed a notable increase in expression in fruits concerning root or leaves, showing great importance in fruit development, that being the reason why both aquaporins were tested in the oocytes system. *CmTIP2;2* had its expression maintained in the root under salinity stress. In that sense, other TIPs like ZmTIP1;2 and ZmTIP4;2 have shown relation in previous studies with Na exchangers and ZmTIP2;2 with Cl solutes transporters in *Z. mays* L. (Yue et al., 2012). Although we cannot confirm this function in melons, we could analyse its implication in other transport functions. In that sense, we proved that CmTIP2;2 was able to transport B, but not water (Chapter III) and, in addition, it is an aquaporin

that, together with other CmTIP2 and CmTIP4 isoforms, is specially constituted for NH_3 transport in a pH-independent function (Chapter I). The common regulation of CmTIP2;2 and CmTIP4;1 is observed in various stresses in roots and leaves and points to a role in nutrients (N and/or B) balance in all tissues. In any case, the great variety of isoforms that are known to be capable of transporting, at least, small amounts of B in oocytes swelling essays highlight the great importance of B for stress tolerance in plants and in melon fruit resistance to cracking. Neither CmTIP1;3 nor CmTIP2;2 were able to transport Si at concentrations they were analysed.

In addition to these two TIPs, three NIPs (*CmNIP1.1, CmNIP2;2,* and *CmNIP5;1)* were selected for oocyte transport assays. In addition to water transport, CmNIP2;2 showed B and Si transport capacity in oocyte tests, showing great versatility in functions (Chapter III). Indeed, it was the only Si aquaporin transporter detected in the melon to date. Its relation to Si uptake became clear in a reduction in Si accumulation in melon affected by salinity, where a decrease in this aquaporin expression in roots and leaves could also be seen (Chapter II).

Regarding those mentioned aquaporins, *CmNIP1;1* showed the same pattern as *CmTIP1;3*, and it is related to B transport in leaves since the same increase in expression in plants under nutritional and high-temperature stresses (Chapter II) was observed. Furthermore, positive results in B transport were found in oocyte tests, clearly indicating that B transport is a fundamental issue related to aquaporin-mediated melon stress tolerance (Chapter III).

Also, *CmNIP5;1* expression was upregulated, together with *CmNIP1;1* and *CmTIP1;3* B transporters, in leaves at high-temperature stress (Chapter II) and in cracked melon fruits (Chapter III), pointing to a possible relation between this stress and the cracking process of melons. CmNIP5;1 aquaporin was firstly supposed to be involved in B transport according to its residue predictions and other NIP5;1 sequences (Chapter I) (Gómez-Soto et al., 2019; Takano et al., 2006), but *Xenopus* oocytes experiments ruled out this hypothesis showing, on the contrary, a great capacity for water transport. Although the results of heterologous expression

analysis should be taken with caution, it seems that water transport is the main function of CmNIP5;1 and it is shown to be regulated in parallel to other Si and B transporter aquaporins. Neither CmNIP1;1 nor CmNIP5;1 were able to transport Si at concentrations they were analysed.

6.4. Nutrient regulation: Ca and Zn.

Several of the most important PIPs were affected by Ca content (Chapter III). *CmPIP2;1*, *CmPIP2;6*, and *CmPIP2;8* have significantly higher levels of expression in Ca+B+Zn in cracked melons (Chapter III), but not in B+Zn cracked melons, pointing to the Ca as a possible regulator of this expression. It is noteworthy that *CmPIP2;6* (Chapter II) showed significantly higher levels of expression in the leaf than in the root, in addition to a significant increase in leaves with high-temperature stress respect control group, being the only PIP in that sense, improving the tolerance of the plants to high temperatures.

Notwithstanding, a significant increase in Ca neither in the roots nor the leaves could be seen (Chapter II), although there were significant increases in the levels of Mg, another divalent cation that could also function as a signaler (Guo et al., 2016). The changes in expression on aquaporins associated with Ca foliar applications have started to be studied. For instance, a recent article described how a sweet cherry aquaporin, *PaPIP1;4*, increased its expression, doubling it in fruit after a Ca treatment (Breia et al., 2020).

In fact, Ca seems to play an important role indirectly, affecting the intake of other important nutrients such as B, Si, or Zn (Chapter III) and it could act as an osmotic molecule. However, some articles maintain that the improvement in cracking with the application of Ca is not due to its osmotic properties (Winkler and Knoche, 2019). This crosstalk between nutrients and aquaporin is widely known (Barzana et al., 2021) and could lead to an accumulation of these elements into the fruit tissue as a mechanism to cope with excessive water intake and, thus, avoiding

the osmotic imbalance that causes the cracking physiopathy (Winkler and Knoche, 2019).

Moreover, it has been proposed that CmNIP2;2 (B and Si transporter) are regulated by Ca, together with CmNIP1;1 (another probed B transporter), being both downregulated when Ca levels were reduced (Chapter II) in leaves. Nevertheless, on the other side, when the Ca treatment was applied (Chapter III), *CmNIP1;1* and *CmNIP2;1* homologue of *CmNIP2;2* (Chapter I) were upregulated by the Ca application which together with significant increases in the levels of this B and Si elements in uncracked melons from our Ca treatment (Ca+B+Zn), point to an accumulation of these two elements by uncracked melons promoted by NIPs Ca-dependent regulation. This fact could improve the resistance and physical properties of melons against cracking since both elements form a structural part and are associated with different stresses to which melons are subjected (Ma and Yamaji, 2006; O'Neill et al., 2004; Rémus-Borel et al., 2005; Rodrigues et al., 2004; Shi et al., 2017).

On the other side, the effect of Zn in aquaporin regulation has been widely described (Barzana et al., 2021; Fatemi et al., 2020) and a Zn regulation of some TIP isoforms was strongly suggested in our studies (Chapter III), including CmTIP2;2. The relevance of Zn in plant resistance to stress and cracking must be further investigated as well as the relation between Ca and the increment in Zn and B intake, how all of them are related to aquaporins modulation and transport functions and the reduction of cracking incidence.

6.5. Final remarks

In summary, the role of different PIPs, TIPs, and NIPs isoforms related to Si, B, and water transport, and the importance of Ca, Zn, and B as aquaporins regulators, reveal the great interconnection between aquaporins and nutrients. Furthermore, aquaporins (mainly, CmPIP1s, CmTIP1;1, CmTIP1;3, CmTIP2;2, CmNIP1;1, CmNIP2;2, and CmNIP5;1) play an essential role when dealing with stresses and

cracking physiopathy, and this opens a new field of investigation with many interesting applications in melon production.

Throughout this doctoral thesis, we have achieved advances regarding the knowledge of the molecular biology and physiology of the melon, we have been able to recognize and analyse all its aquaporins and study their behaviour in different tissues and stress situations. Light has been shed on water regulation and the important relationship between nutrients, water, and resistance to stress and cracking physiopathy in this plant, characterizing its effects and the mechanisms involved. The functions of the different isoforms of aquaporins in these processes have been elucidated.

The research as a whole will serve as the basis for multiple applications, having pointed out important molecular markers for the selection of varieties as well as fundamental nutritional factors to take into account in real field conditions. Specifically, the great importance of Ca, B, and Zn in melon cultivation has been elucidated to prevent the incidence of cracking.

Conclusions

7. Conclusions

I. The melon has 31 aquaporins, 12 PIPs, 8 TIPs, 8 NIPs, 2 SIPs, and 1 XIP, these sequences being highly conserved, with very high percentages of similarity to other species such as watermelon or cucumber.

II. The aquaporins with the highest expression values in the roots, leaves and fruits of melons were *CmPIP1;1*, *CmPIP1;2*, *CmPIP2;2*, *CmPIP2;10*, *CmTIP1;1*, *CmTIP3;1* and *CmNIP5;1*.

III. Other aquaporins had high expression tissue-depending values. In the roots are *PIP2;6*, and *NIP5;2*, in the leaves are *CmPIP2;3*, *CmPIP2;4*, *CmNIP2;1*, and *CmNIP5;2*, and in the melon fruit are *CmPIP2;3*, *CmPIP2;5*, *CmPIP2;6*, *CmTIP1;3*, *CmTIP2;2*, and *CmNIP6;1*. *CmXIP1;1* was tissue-specific in melon fruit.

IV. Under the abiotic stresses (salinity, micronutrient deficiency and high temperature), a general decrease in the expression of the aquaporins was observed in root (*CmPIP2;1*, *CmPIP2;5*, *CmPIP2;6*, *CmTIP1;2*, *CmTIP1;3*, *CmTIP2;1*, *CmTIP3;1*, *CmTIP5;1*, *CmNIP6;1*, *CmNIP7;1*, and *CmSIP2;1).* Among them, *CmPIP2;1*, *CmPIP2;5*, and *CmPIP2;6* strongly correlate with a decrease in L_o.

V. Melon is tolerant to the salinity applied thanks to a wide regulation of the hydric parameters based on aquaporins regulation as *CmNIP2;2* in the root, a proved water transporter, decreasing the water intake, a significant increase in *CmTIP1;1* in the root, and *CmPIP1;1* and *CmNIP6;1* in the leaf increasing the general water turgor and maintaining growth.

VI. Melon was strongly affected by the micronutrient deficiency with a wide diminution of nutrient uptake and plant growth, in consonance with aquaporins expression. In leaves, *CmTIP1;1* decrease denotes a decreased exchange of molecules between the vacuole and the intracellular space. A significant increase in the expression of *CmPIP2;2* and *CmNIP5;1* in the root and *CmTIP1;3* and *CmNIP1;1* in the leaf, favour the transport of both, water and B, respectively.

VII. Melon plants are tolerant to high-temperature stress, showing no changes in physiological parameters but regulation of aquaporins expression with increases in *CmPIP1;1, CmPIP1;2, CmPIP2;2,* and *CmTIP1;1* in the root and *CmPIP2;6, CmTIP1;3, CmTIP2;1, CmNIP1;1,* and *CmNIP5;1* in the leaf, maintaining the water flow and growth.

VIII. In the three stresses, it could be seen a decrease in K, Fe, Mn, Mn, and Zn in the root. Only Ca, in the roots, and Fe, in the leaves, were stable in all treatments. Under salt stress, it could be seen the typical pattern of competition between K and Na. In micronutrient deficiency, a general decrease was found, highlighting unchanged levels of Ca, in addition to a significant increase in B and Mo levels, pointing to these elements as relevant to this stress. Finally, in high-temperature stress an increase in B, Mg, and Si levels in both, roots and leaves, seems to be a clue to resistance to temperature.

IX. Both foliar treatments proposed, B+Zn and Ca+B+Zn, applied for three weeks, once per week, were able to reduce significantly the incidence of cracking (17.61% incidence in control vs. 8.17% in Ca+B+Zn and 5.71% in B+Zn) at the fifth week of the field study.

X. Ca enhances the absorption of other elements as Ca+B+Zn treatment, increase the concentration of Ca, B, Si, and Zn in the pulp of uncracked melons while it does not happen in control or B+Zn treatment.

XI. Cracked melons increased the expression of aquaporins such as *CmPIP1s, CmPIP2;8, CmPIP2;10, CmTIP1;1, CmNIP2;2,* and *CmNIP5;1* aquaporins. Similarly, the treatments also affected the expression patterns, mainly by decreasing the expression levels of some aquaporins like *CmPIP1;1*, several *CmPIP2s, CmTIPs,* and *CmNIP5;1*. In both scenarios, most of the aquaporins are related to water transport.

XII. Water transport is involved in the appearance of cracking since important aquaporins such as CmPIP1;1, CmPIP1;2, CmTIP1;1, CmNIP2;2, or CmNIP5;1, which are water transporters, have significant increases in their expression in cracked melons.

XIII. The aquaporin CmNIP2;2 has been discovered as a transporter of B and Si and proposed as involved in cracking, since it was the only aquaporin, together with CmNIP5;1, that had a significant increase in their expression in untreated cracked melons compared to non-cracked ones.

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Annexe

9. Annexe. Melon aquaporin sequences

PIP sequences:

>NM_001393778.1 Cucumis melo aquaporin PIP1-1 (PIP1-1), mRNA
ATTTCCTCACTGCTACTAAAGACAGAACCAACGTCTTCGTGAGCACAGATAAGTTTTTCC
GAAATGGAAGGGAAGGAGGAAGATGTTAGACTTGGAGCTAACAAATTCAACGAGAGACAGC
CGATTGGAACGGCGGCCCAGAGTCAAGATGACGCTAAGGACTACAAGGAGCCACCGCCGGCC
CCGTTGTTTGAGCCAGAAGAGCTCACTTCATGGTCCTTCTACAGAGCTGGAATTGCCGAGTTTT
TCGCTACTTTCCTCTTCCTCTACATCACCGTTTTGACCGTCATGGGTGTCGTCCGGTCCAAGAA
TATTGAGGGGAACACGTGTAAGACAGTCGGAATTCAGGGTATTGCTTGGGCATTCGGCGGTAT
GATTTTTGCTCTGGTTTACTGTACTGCTGGAATTTCCGGTGGGCACATTAACCCGGCGGTGACT
TTCGGGCTGTTTCTGGCGAGGAAGCTGTCGTTGACTAGGGCGATTTTCTACATGGTGATGCAGT
GCCTTGGTGCCATCTGCGGTGCTGGTGTGGTGAAGGGGTTCCAACCCAAGCTCTACGACACCC
TCGGCGGCGGAGCTAACATTGTGAGCGAGGGATACACCAAAGGAGACGGCCTTGGCGCTGAG
ATTGTTGGTACTTTCATCCTTGTTTACACCGTCTTCTCGGCCACCGATGCTAAACGTAGCGCCA
GAGATTCACATGTTCCGATATTGGCACCATTGCCAATCGGATTTGCAGTGTTCTTAGTTCACTT
AGCCACCATTCCGATAACGGGCACCGGCATCAACCCAGCCCGGAGCTTGGGAGCCGCCATTAT
TTACAACAACGAGAAAGCATGGGATGATCACTGGATATTCTGGGTGGGACCTTTCATCGGCGC
TGCTCTTGCAGCTCTGTACCACCAAGTTGTAATCAGAGCCATCCCTTTCAAGTCCAAGTGAAA
ATCTTATCTAAATCCATGATGCTTCTTCAATCAATCACAGCCGTCCGATCCGAAAAGAAGAAA
AAAGAAGGCTGTTTTCCCCTTTTGTGTTGTATTTTTTTTTCTCTAATGGTTTTTAAAATTTCAAC
TTTGGGACCCAATTGTTAAGGGAGTGCAGTGTTATCTGTAATTATATGTGTGGCTATGAAGCTA
TGTAATGGTTAATTTCCCGAAAAAAAAAGTTGAAATAATGATACCCACTCTCCTTTTACCTGCT
TTA

>XM_008439071.2 PREDICTED: Cucumis melo aquaporin PIP1-2 (LOC103482758), mRNA
GCCATCCTCCTCCTTCTACATTCTTCCCCAAAAGCAAAAGCAAAAAAAACCCATTAACAGGAG
AGAGAAATCTCATTTTAGAGTGAGAAAAAGCCTCCATTGTTACATTTTCTAAGTAAAACTCAG
CAAAGAAGGAAATGGAGGGGAAAGAAGAGGACGTTAAGCTAGGAGCAAACAAGTTTTCAGA
AAGGCAGCCAATTGGCACATCGGCGCAGACGGACAAGGACTACAAAGAGCCACCGCCGGCGC
CATTGTACGAGCCGGGCGAGCTGACGTCATGGTCGTTTTACAGAGCCGGAATCGCGGAGTTTA
TGGCCACTTTCTTGTTCCTCTACATCACCATCTTGACGGTGATGGGAGTCAGCCGATCTCCGTC
CAAATGCAACACCGTCGGCATTCAGGGCATTGCTTGGGCCTTCGGCGGCATGATCTTCGCCCT
CGTCTACTGCACCGCCGGCATCTCCGGTGGGCACATTAACCCGGCGGTGACCTTCGGGCTTTTC
CTGGCCAGAAAACTGTCCCTCACAAGAGCATTATTCTACATCATAATGCAATGCCTCGGCGCC
ATCTGCGGCGCCGGCGTGGTCAAGGGCTTCGAGGGGTCCACCTACGTGCAGAAGCTGGGTGGT
GCCAACTTCGTGGCCTCTGGCTACACCAAGGGCAGCGGTCTGGGTGCTGAGATCGTCGGTACC
TTCGTTCTCGTCTACACCGTCTTCTCCGCCACCGATGCTAAGAGAAATGCCAGAGATTCTCACG
TCCCTATTTTGGCTCCTCTTCCCATTGGGTTTGCAGTGTTTTTGGTTCATTTGGCCACCATTCCC
ATCACCGGAACCGGCATTAATCCGGCCAGGAGTCTCGGAGCTGCAATCATCTACAACAGGCA
ACACGCTTGGGATGACCATTGGATCTTCTGGGTGGGACCTTTCATCGGAGCTGCTTTGGCTGCT
ATTTACCACCAAATCATCATCAGAGCCATTCCATTCAAGGCCAGAGCTTGAAGATCAGAAGAA
CAGTTTCAAAATTCTTAATATCATTATTATCATTTCCTAAGAGGTTATCTTTGGAATATATCATC
TTCTTTTTTTCATTTCTTTCTTAATATGTGTGTTCTTCTTTATCTCTTTTTTGGTATCTGTAAACTT
CGTTTATTTTGGTGTTCTTGAGAGAAACTGAGATGGGTTTGTGTGTAAATTAAGTTATCTGTCT
TAATTATTAGTGGATGTTGTAATTTTCAAGTTATGTAATGGATTATATATTAATTCTTTTGTGCA
ATCA

>XM_008451030.1 PREDICTED: Cucumis melo aquaporin PIP2-1-like (LOC103491188), mRNA
TCCATTACTCTGTTTCTCCATCAGCAAATTCTCCTCTGTTCTTCTAACACTCAAATTTGATAATT
GAGATTGTTTGATTTATAGAGCTATGCCGAAGGATATTGAAGCTGGAGGGCATGGTGGGTTTA
GTGGGAAGGATTACGAAGACCCACCGCCTGCGCCGTTGATCGACGCACATGAGTTTGCTAAGT
GGTCATTTTACAGAGCCATTATAGCTGAGTTTGTTGCTACTCTTTTGTTCTTGTATGTTACTGTT
CTTACCGTTATTGGGTATAAAGTTCAGAGTGATGTTAAGAATGGAGGGGAGATTTGTGGCGGC
GTTGGAATTTTGGGTATTGCTTGGGCCTTTGGTGGCATGATTTTTGTCCTCGTTTACTGCACCGC
GGGGATTTCTGGGGGACATATAAATCCGGCGGTGACTTTTGGGCTGTTTTTGGCTCGTAAGGT
GTCGTTGGTGAGAGCCATATTGTACGTGGCGGCTCAGAGTTTAGGCGCCATTTGTGGCTGTGC

ATTGGTGAAGGCGTTCCAAAACGGTCATTACACCGAGTACGGTGGTGGAGCCAATTCCCTCGC
CGACGGCTACAGCACCGGCACTGGATTAGCCGCTGAGATCATCGGAACCTTCGTTCTCGTTTA
CACTGTCTTTTCCGCCACCGATCCCAAAAGAAATGCCAGAGACTCCCACGTCCCCGTTTTGGC
GCCACTTCCAATTGGGTTCGCGGTGTTCATGGTTCATTTGGCCACGATTCCAATCACCGGAACC
GGCATCAACCCAGCTCGAAGCTTTGGAGCTGCAGTTGTGTTCAACCGATCCAAGCCATGGGAT
GATCAATGGATATTTTGGGTTGGACCTTTCATTGGAGCTGCCATAGCTGCAATTTATCATCAAT
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TAATTAAGCCTAATAGTTGTGTGCTATAAGTATTTTGGCAAAAATGCCAATTTGGTTTGTGTTC
TGTAAAAAAGAAAAAAAGAGCATTGGCTTTTTATTTTATATGCCCATATGATCTGCTTTTGCTT
TCGTGGTTTTTGCTCTCTTTTTATGTAATTACGAGATGTTTCATGATGTTTATAATTAAAGTGTG
AATGCATCCTCAATTTCTTGTTTTAA

>XM_008451033.2 PREDICTED: Cucumis melo aquaporin PIP2-2-like (LOC103491189), mRNA
CCTCCTTCACTTTTCCTATAAATCTTTTTCATTTCCATCCATAACCCATCGTCCCTTTTCTTCTCT
ATCTCTTCATTTTCTCCAGCCATGGCCAAAGATTCCGATGCCGGAGCCTTCGCCGCCAAGGACT
ACCACGACCCTCCACCGGCCCCTTTCATCGACCCCCACGAGTTTACTCAATGGTCATTTTACAG
AGCCATCATCGCCGAGTTCATCGCCACTCTCCTCTTCTTGTACGTCACTGTTCTCACTGTCATTG
GCTATAGTAGCCAGTCCGACATCAAACATAACGGCCAAATCTGCGGTGGTGTCGGCATTCTTG
GCATCGCTTGGGCTTTCGGTGGAATGATCTTCGTTCTTGTCTACTGTACTGCTGGAATTTCAGG
TGGACATATCAATCCGGCGGTGACATTTGGGCTGTTTTTGGCTCGGAAAGTCTCGTTGGTGAG
GGCTGTTCTGTACATGGTTGCTCAATGCCTTGGTGCTATTTGTGGATGTGCTTTGGTTAAATCG
TTTCAAAAGGGTCTTTACATTCGTTACGGCGGTGGAGCCAATTCTCTCGCCGACGGCTACAGT
ACCGGCACTGGCTTAGCCGCTGAGATCATCGGAACTTTCGTTCTTGTCTACACTGTCTTCTCTG
CTACAGATCCCAAGAGAAGCGCTAGAGATTCTCACATTCCAGTTCTAGCTCCTCTGCCAATTG
GGTTCGCCGTGTTCATGGTTCACCTAGCCACCATTCCAGTCACCGGCACCGGTATCAACCCAG
CTCGAAGCTTTGGTGCTGCGGTGGTTTTGAACGAGAGCAAGCCATGGAATGACCATTGGATAT
TCTGGGTTGGGCCATTCATCGGAGCTGCAATTGCTGCATTTTATCACCAATTTATTTTGAGAGC
TGGAGCTGTCAAAGCGTTGGGATCTTTCAGGAGTACTCAAAGTGTTTGATGATTTTGTTGATGA
GAAGAATGGGGGTTTGGCTTTGGATCTAAAATGTGTGATTAGGGAAGACATTTGAGTTTTAGT
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TGTTGTTCAATCCTTTTTACTTTTTAAGCACATATTTTGAAGGTTTTATTTTTAATTATTGCAAA
AGAAAGTTGTTTGGTTTAAGAAATGGATGTAGGGAAATTGTGTTGGTTCTTCTAAGAACTTTA
AATCCAAGGACATTGGCATCCCAT

>NM_001393779.1 Cucumis melo aquaporin PIP2-3 (PIP2-3), mRNA
TGGGGCGGCAACAAAATTCCAATACATATATTTGAATTCACAAATCCACTCCTCTAAACATCC
TTTGTATAAATAAATCCACCACTTCATCTCTAACATCCATCACTCCCCTCTACAACTCTTCTGTT
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CTTGAAGCCGGTGGATTCGCCGTCAAGGATTACCAAGACCCACCTCCAGCCCCATTGATCGAC
GCCGAAGAGTTAACTCAATGGTCTTTTTACAGAGCTATCATTGCCGAGTTCGTCGCCACTCTTT
TGTTCTTGTACGTCACCGTTCTCACTGTCATTGGCTACAGTAGCCAATCCGACACCAAAAGCGG
CGGCCAAATCTGTGGCGGCGTCGGCATTCTCGGCATTGCTTGGGCATTTGGTGGCATGATCTTC
GTCCTCGTTTACTGCACTGCTGGAATCTCCGGAGGGCATATTAACCCGGCGGTGACTTTTGGGT
TGTTCTTGGCTCGGAAAGTATCTTTAATTAGAGCAATTCTTTACATGGCGGCTCAATGTTTGGG
CGCCATTTGTGGCTGCGCTTTGGTGAAATCATTCCAGAAGGCTCTTTACAATGGATACGGAGG
TGGAGCCAATTCTCTCGCCGATGGGTACAGCACCGGCACCGGCTTAGCCGCGGAGATTATCGG
AACTTTCGTTCTTGTTTACACCGTCTTCTCCGCCACCGATCCCAAGAGAAATGCTAGAGATTCT
CACGTTCCTGTTTTGGCTCCACTCCCAATTGGGTTCGCGGTGTTTATGGTTCATTTGGCCACCAT
TCCGATCACCGGCACTGGAATCAACCCAGCTCGAAGTTTTGGAGCTGCCGTGATCTATAACAA
AGACAAGGCATGGGATGACCAGTGGATATTTTGGGTTGGACCCTTCATTGGAGCTGCGATTGC
TGCAATTTATCATCAGATAGTGTTGAGGGCAGGAGCAGTTAAAGCTCTGGGTTCTTTCAGAAG
TTCGACTGCCGTATGAATGTGATATAAATCTGCATGAATGAATGTGGAAGTTGTTTAGAAAAA
ATCCCAAAGGCACTTGGTTTTTTGAAGTGTGCCCTTGTTCTATCCTTCCAGTTGCTGTGTTTCTG
TTTTGCTTTTACTCTGTTATTCGTCCCCCACAAGTTATATGTAATATTGGGCTCTGTTTTAATGC
AAAGTTTGATCCAATCAAGTGGGTCAGTTTCTA

>NM_001393782.1 Cucumis melo aquaporin PIP2-4 (PIP2-4), mRNA
AAATCCATCTCCTTCCTTCTGGTACTTTCTTCCCCACTCCTTAAATACCCTTTTCTTCCCCCTTTT
TCCTCCTCACTACCACTTTCCCACTCCCAATATTTCTCATCCCCAAATTCTCCACACCCAACAA
CTCCAAATCCACAACAATGGGGAAAGACGTTGAAGTTGCTGAGCAAGGTGAATTCCAAAGCA

AAGACTACCAAGACCCACCTCCCGCCCCTCTCATCGATCCCGAAGAGCTCACTAAATGGTCTC
TTTACAGAGCCGCCATCGCCGAGTTCATCGCCACTCTCCTCTTCCTTTACGTCACCGTTTTGAC
CGTTATCGGTTACTCCCACCAGAGGGACTCTACCCCCGACCCCTGTAGCGGTGTTGGCATTCTC
GGTATCGCTTGGGCCTTCGGTGGTATGATCTTTATCCTTGTTTACTGCACCGCCGGTATCTCCG
GTGGCCACATTAACCCTGCTGTCACTTTCGGCCTTTTCTTGGCTCGTAAAGTCTCTCTGGTTCGT
GCTGTATTGTACATGCTTGCTCAATGTGCCGGTGCTATTTGCGGCTGTGGCTTGGTTAAAGCCT
TCCAATCTGCTTATTATGTTAGATATAACGGTGGTGCTAACATGCTTAGTGATGGTTACAACAA
AGGAACTGGGTTGGGTGCTGAAATTATTGGGACTTTTGTTCTAGTTTACACTGTTTTCTCTGCT
ACTGATCCCAAGAGAAACGCCAGAGATTCTCACGTTCCTGTTTTGGCTCCTCTCCCAATTGGGT
TTGCTGTGTTTATGGTTCACTTGGCTACAATCCCCATTACCGGAACTGGAATTAACCCTGCTAG
AAGTTTCGGAGCTGCTGTCATTTTCAACAAAGAGAAAGCCTGGGATGATCAATGGATCTTCTG
GGTTGGACCATTCATTGGAGCTGCAATTGCTGCAATTTACCATCAGTATGTGCTCAGAGCTGG
CGCCATTAAAGCTCTTGGATCTTTCAGGAGCAACGCTTGAAAATCCTTCAATGGAAATATATTT
AATTGGTTTCTCTCAGATTGTGTTTTGTTTTTTGTTTTTTTGTTTTTGGGGTTCTCCTTTTTCTCTC
TTCTCAATAGACTTTCGATGGTTGAATCGGAACTCAATTCTTGCGGCTGCTCTGCTCTCGTCTG
CTTTGGTCCGTCGTGGAATTAAGTTCTACAAGAAGTTTTAATTGTTGGCCCTGGAAATTGTACA
TATTTCCATTTCTCTCCCCTAAAAGAAGAAAAAGAGAAAAAAAAGAAAGAAAAAGAAATTGG
GGCTCATTCTCATCCTATGTATTCATGTATTTTTCTTTTTGTGTTTCATCCTTATCCTTTGTTTTTT
ATTTAATAAAGGAATCTCCTCTATTATGATGTTCA

>NM_001393787.1 Cucumis melo aquaporin PIP2-5 (PIP2-5), mRNA
ACCATTTCCCATTTCCTCACCTTGCTACCAAACTACCTTTACACACTAACCAACGCAACAAAAA
ACCTCCCTCTCGAATTTCCGCCGTTCCCTCGCCGGGATTTCATAACAATGTCCAAAGAAGTGAC
GGAAGAGGGACAGTCCGGCCTCAGAAAGGACTACGTCGACCCACCCCCGGCTCCACTCATCG
ACGTCGCCGAACTCACCCTCTGGTCCTTCTACAGAGCCCTTATCGCTGAGTTCATCGCCACTCT
CCTCTTTCTCTACGTCACAATCGCCACCGTCATCGGCAACAACAAACAGACCAAAATGTGTGA
TGGCGTTGGAATCCTCGGAATCGCCTGGGCCTTCGGTGGCATGATCTTCGTCCTCGTCTACTGC
ACCGCCGGAATCTCCGGTGGTCATATTAATCCAGCGGTGACATTCGGGTTGTTCCTTGCGAGG
AAAGTGTCGCTGATCAGAGCGTTTGGGTACATGGTGGCGCAGTGCGCCGGAGCCATTGTTGGC
GTTGGGTTAGTCAAGGCTTTCATGAAGCATGATTATAACAACAACGGCGGCGGAGCCAACGCC
GTTAATTCTGGTTACAGTAGAGGAACAGCTCTTGGTGCTGAGATTATTGGAACTTTCGTTCTTG
TTTACACTGTCTTCTCCGCTACTGATCCCAAGAGGAGCGCGCGTGATTCTCACATTCCTGTTTT
AGCTCCATTGCCAATTGGGTTCGCCGTGTTCATGGTTCATTTAGCAACCATTCCCATTACAGGA
ACCGGAATCAACCCGGCCAGAAGCTTCGGCGCCGCCGTCATCTACAACCGTGAAAAACCCTG
GAATGACCACTGGATCTTCTGGGTGGGTCCGTTCGTCGGAGCATTAGCGGCGGCAGCGTACCA
CCAGTACATTCTCCGGGCAGCCGCCATTAAAGCTTTGGGATCATTCCGCAGCAACCCCACAAA
CTGAGAAGGAAGAAGATGATGGTAGAAGTGGAGCATGTTTTTAGAATTATTCATTATTAATTT
GTGTGGTTATGTGTGATGATGAATGAGATTATGAGGAGGATGAAGGGAAGGGATTGTCTTTGA
ATGTTTTAATTTTATTTAATTTTTATAATCTTTTGTTTGTTTAGTTTATTTTTGGCTGTGTAATAT
TATGATTATCTCTTTCCTTTTGTTCATTATAAGACAATTTGGCCTTCAATTTTCTTA

>NM_001393783.1 Cucumis melo aquaporin PIP2-6 (PIP2-6), mRNA
AGGGTATAGATGCCCTCTCAAAAGGTATACATGAAACCCTAAGGAATAGATTATTCCAATTCC
CAAGCTCTCTCTATACATAATAACACAAAACCAAACAAACTAAGTATATATTTATTACCATTTC
TAACTTCGTAGCAATAATAATTCATAACTCTCCTCTCCTCTCCTCTCCTCTCATCGAAAC
AGAGCAATGTCCAACAACATTGATGGAAGAAGCAATGTCAAGGACTACCAAGATCCACCTCC
CGCTCCCCTCATCGACTCCGACGAGTTCTCTCAATGGTCATTTTACAGAGCCATTATCGCTGAG
TTCGTTGCCACGCTTCTCTTCTTGTACATTCTTGTTCTCACTGTCATTGGCAATGCCAGACTCTC
CGACACCAATATCTGCGGCGGCGTCGGCGCTTTAGGCATTTCCTGGGCCGTCGGCGGCATGAT
CTTCGTCCTTGTTTATTGCACTGCCGGAATTTCTGGTGGCCATATTAATCCGGCGGTGACGTTC
GGTATGCTGTTAGCTCGAAAAATCTCCCTAGTCAGAGCTTTGTCTTACATTTTGGCTCAATGTT
TGGGCGCAATTTGTGGATGTGGTTTAGCTAAATCATTACAACAGACTTATTACGTTCAGTACAA
CGGCGCAGCCAATATGGTGTCAGATGAGTACAGCATCGGCACCGGCTTAGCCGCAGAGATAA
TCGGAACTTTTGTTCTTGTTTACACTGTCTTCTCCGCCACCGACCCCAAAAGAAACGCTAGAGA
TTCTCACGTCCCAGTTTTGGCACCACTCCCAATTGGGTTCGCTGTGATTATGGTTCATTTAGCT
ACCATTCCGATCACCGGCACCGGCATCAACCCAGCTAGAAGCTTAGGAGCTGCTGTGATCTTT
AACAAAGCCAAGGCCTGGGATCATCATTGGATCTTTTGGGTTGGGCCATTCATTGGAGCTGCC

ATTGCTGCAATTTATCATGTAGTGATAATAAGGGCAGGAACCATTAAAGCTCTGGCTTCCTTC
AGAAGTTCATCTGCTCTATAAATCTTATCCCAATCTTGTTTGTTCCAAAAAAAAAAAAGAAAAA
AAGTGAACTTTTTAAGGCACTCTGTTTTCCCCTGCCATTTTATGTAATTCTGAATTTTGCTTTTT
TGTGCTGAACTAATGAAGTGTGTTAGATGTTTA

>NM_001393784.1 Cucumis melo aquaporin PIP2-7 (PIP2-7), mRNA
CTCAAACCTCTTTCCCCTCCTCTTTTTCTTATTACTTTTTTGCTCCCCCTATGGCCAAAGACGAC
GTCACGGCGGCGCGAGGGTTCTCCGGAAAGGACTACCACGACCCTCCTCCGGCCCCGCTCTTC
GACGCCGCCGAGCTCGGCAAGTGGTCCTTTTACAGAGCCATCATTGCTGAGTTCATTGCCACT
CTTCTTTTTCTCTATGTCACTATCTTGACTATTATCGGCTACAACAGCCAGACCGATACCAACA
AGCCCGGCACCAACGCTTGCGATGGGGTCGGCATTCTCGGCATAGCTTGGTCCTTTGGCGGCA
TGATCTTCGTCCTTGTCTACTGCACTGCTGGCATTTCAGGAGGGCACATAAACCCGGCGGTGA
CGTTCGGGCTGTTCTTGGCTCGTAAAGTGTCGTTAGTGCGAGCAGTAGCGTACATGATAGCTC
AGTGTTTGGGGGCCGTGTGCGGTGCGGGGCTAGTAAAAGCCTTCCAAAAGTCTTACTACAACA
AGTACGGCGGCGGTGCGAACCAACTCGCCCATGGCTACTCCAAAGGCACCGGCTTGGGAGCT
GAAATCGTCGGTACCTTTATCTTGGTTTACACCGTCTTCTCCGCCACCGATTCCAAGAGAAATG
CCAGAGACTCCCATGTTCCTGTATTGGCTCCACTCCCAATTGGGTTTGCTGTGTTTATGGTTCA
CTTGGCCACCATCCCCATCACCGGCACCAGCATCAACCCCGCAAGAAGCTTCGGCCCTGCTGT
TATCTTGAATAGGGAAAAACCTTGGGATGACCATTGGATATTCTGGGTTGGGCCATTTGTGGG
AGCCGCCATCGCAGCGTTCTACCACCAGTTCATATTGAGGGCCGGCGCCGCCAAAGCTCTCGG
CTCTTTCAGGAGCAACCCCTCCGTCTGATCGCCATTCCATAAGCAAAAGACTACTTTTAAAGTC
TTTTTTTTTTTTCTTTTTCTTTTTAACTTTTCGTTGGGGCCAATTGTTTAAATATTGTTTGTTTTTC
TTTCTTTTTAGTATTTTAACTTTTTTAAGTTTGTTGTATTGTAATTTGTAATGTAACCACTTGGCT
TACTAATGTTCATTTTTTATGTTCTTAAATTAT

>NM_001393785.1 Cucumis melo aquaporin PIP2-8 (PIP2-8), mRNA
AACAATAAATAAATAAATAAATAAAAGAAATTGGAGTAATGTAATAATAATTAGAAAAAGAG
GATGGACAAATGGTTCTCTCAAAATCGCTCTCATTCTAATCCTAAGACTAAATTTGGAGAGAA
ATCATTTTGTGGCGGAAATTTTCAAAAATCAATTCCAACGAACGAACCACAAATTCCTTCATA
GAGAGATGCGGATTAACACAGACAAAACCAAAGAAGTGTTCGGATGTCAAGAAATTCAAACA
AAATCCCTTAAATCTCATCAATCATTCAACTATCCATCTCCAAATTTCCCCATTCCCGAACTCA
CAATCGAAGCCCTAACAAAAATTTCACTCACTTCACATTCACTGAGATCCTGCACCAATGGCG
AGCAGCGACATCGAAATCGGAGGCCGGGGGGCGGCGTCGAGGAAAGACTATTTCGATCCGCT
TCCGGCGCCGATGATCGACATGGAGGAGTTCGCGAAATGGTCGTTTTACAGAGCTATCATTGC
TGAGTTTGTGGCGACTTTGTTGTTTCTGTACGTCGTCGTTTTGACGGTGATCGGAAATAGTAGC
CAGACGGACCCGCTCAACGGTGGGAATGTATGCGCCGGAGTTGGCCCCGTAGGAATCTCGTG
GGTCTTCGGGGGTATGATCTTCGTCCTCGTCTACTGCACCGCCGGTATCTCTGGAGGGCACATA
AATCCAGCAGTGACATTCGGAATGTTTTTAACAAGAAAGATATCGTTGGTCCGAGCGTTGCTC
TACATCATAGCTCAGTGCATAGGGGCCCTATGTGGGTGCGCCTTGGTCAAGACATTGCAGAGA
GATCGTTACAACCACTACGGCGGTGGTGCTAATCAGCTCGTTGATGGTTATAGCCGAGGCACT
GGCCTTGCTGTTGAGATTGTGGGCACCTTTGTTCTTCTGTATACCGTCTTCTCCGCCACTGATCC
CAAACGCAATGCTAGAGATTCCCATGTTCCTGTCTTGGCTCCACTCCCCATTGGCTTTGCTGTC
TTCATGGTTCACCTCGCCACCATTCCCGTCACCGGCACTGGCATTAACCCTGCTCGAAGCTTCG
GTGCTGCCGTCATCATCAACAATCATAAAGTTTGGAAAGATCATTGGATTTTTTGGGTTGGGCC
GCTAATTGGATCAACAATTGCTGCAATGTATTATCAATATGTTCTTCGAGCCTCCGCCGTGAAA
GCAATCGGATCTTTTAGAAGCTCACCGCCAACTCATTAAAATCATGGACATAAGTTTTTGTTTT
TTTTCTCCCTATCTTATTATCTTTTTTTTTAGTAGTAGAGAAGAATACAAATGAGAAGATGATTT
TTATATAATCTTTTTCCCTCCCCAAGTAGAAAGGTAGAACAGAAGTGTTTTTTTCCCCAAAGTG
AATTCTTTGTTTAAAATATGATTTTGTAATTTTGGGTAGTGAATTGCTAGATTGGTGTACTATTG
AATGAATGGTTTTAGTTCTCAATTTTTGAA

>NM_001393786.1 Cucumis melo aquaporin PIP2-9 (PIP2-9), mRNA
AACTAATCGAAGCCATTGGAAGTTTTTACCGATCATGGATTCCACTTTACATCCCAAGCATATT
TTCTTCATCTCTTAATCTCCTATTTATTATCATTCCTTAATTCAAACATTACTAACTCACTCACT
CACTCACTCTCTAATTGCCCACCCCCTCCCCTGTTTCTTCCCATAGTTAATAATGTCTAAGGATC
TTGATCCTGGTTTTCCCACCGGCGACTACTTCGATCATCCTCCGGCTCCCTTCTTCGACTCTGAA
GAGCTCTTGCGGTGGTCCTTTTATAGGGCTGTTATTGCCGAGTTCATTGCTACTCTTTTGTTTTT
GTATGTTGGAGTTCTTACTGTGATCGGAAGCCAAAGTCAGGATTCTGCCGCGGTATGCGGCGG
CGTTGGTGTTCAAGGTATTGCTTGGGCGTTCGGCGGCACCATCTTTGTTCTCGTTTACTGCACC

GCCGGCATTTCTGGAGGACATATAAATCCAGCGGTAACATTTGGTCTGTTCTTGGGTCGGAAA
GTTTCATTGGTTAGAGCTGTGTTGTACATAATGGCTCAGTGTTTAGGAGCCATTTGTGGGTGTG
GTCTAGTGAAATCATTAAAGAAGGCTAATTTCAATACCTTTAGCGGTGGAGCCACCGAACTCG
CCGACGGCTTCAGCCCTAGTGCTGGTCTTGCTGCTGAGATCATCGGAACCTTTGTTCTTGTATA
CACTGTCTTCTCTGCTACCGATCCCAAGAGAAAAGCTAGAGATTCTCACGTTCCCGTTCTAGCG
CCGCTCCCAATTGGGTTTGCGGTGTTCGTGGTGAATTTAGCGACGATTCCGGTGACCGGCGCC
GGCATTAACCCAGCTCGAAGCTTCGGCTCAGCAGTGATGTTCAACAACCACAAAGCTTGGGAT
AACCATTGGATATTCTGGGTTGGACCTTTCATTGGAGCTGCAATGGCTGCAACTTACCATGAAT
TTGTTTTGAGAGGAGGAGCAGCTAAGACCTTGAAATCCTTCAAAACTTCCTCAGTTTAATAATT
CTTCTTCCTTACACTTCATGCAAAATCCCCACTCTAACTTCCTTTTTCTTTTCCAATGAAACAAA
TAATCTACCTCTCATCTGAAAATTTAGAAGAAGCAATCTTTTGTAATTGGGTAATCTCTTTAAT
TGCAATCAAAAAAACCCTTTGAAATTTGAATAACCAAGTATTATTAATAGGTTAAAAAATCAC
TTCCATGTCCCGAAACCTTTGTAGATTAGTAACCATTAATGTGTGAGGAATTGAGAGCTAAGTT
TTCAATATTATTCAGCTTAAGATTGAGAA

>XM_008458254.2 PREDICTED: Cucumis melo probable aquaporin PIP2-10 (LOC103496419), mRNA
TACCAACGCCATTGATTATTTATTTGAAGCATGAAAAGGAATTTGTCGTTACTGTAGTAAGTGG
CATGCACAGTGAAGGCACTGTTCTGAGTGGGTCAGAAGGAACGAACGACAAAGGTACGGAAG
CAACCAAAGGCAATGGCAAGTGGGTAGGCGGTGGTGCCCAACTCAGGCGGAGCCGCTGAAAA
TCACTGCTGCTTTTCTCTTATTTTCCTTCAATTTCTTCTTCATTAAATGCCGCTATTCGGATCGCC
AAATTGCACCACATTTCTTTATTCAAAGTCTCCTCTAGGGCTTTGGTTTTGGTTTCAATTTTCTG
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CTTGTTTACTGTACTGCTGGAATTTCAGGGGGCCACATAAACCCAGCAGTGACATTTGGGCTTT
TGTTGGGCCGAAAGGTGTCACTTGTGCGAGCCGTGATGTACATGGTGGCCCAATGTTTGGGGG
CCATCAGTGGAGTTGGGCTTGTCAAGGCCTTCCAGAAGGCCCACTTCCAGAAGCACGGCGGCG
GGGCTAATGGGGTTTCCGATGGCTACACCATCGGCACTGGACTGGTCGCCGAGATTGTTGGCA
CTTTTGTGTTGGTGTACACCGTCTTCTCCGCCACCGACCCTAAACGTAACGCCAGAGATTCCCA
TGTTCCGGTTTTGGCTCCACTTCCAATTGGGTTTGCAGTTTTCATGGTTCATTTGGCTACAATTC
CAATCACGGGCACTGGAATCAACCCAGCTAGAAGCTTGGGATCCGCCGTTATTTTAAATGATC
AAAAGGCTTGGAATAACCATTGGATATTTTGGGTTGGACCATTTCTTGGAGCAGCCATTGCAG
CTTTCTACCACCAATTCATCTTGAGGGCAGGTGCTGTTAAAGCTCTTGGTTCATTCAGAAGCAA
CTCACATGTGTGATGATAATTAGAAATGTTGTTTTTAATTAAGCTACCTCTTTGGAGATTAAGG
TTCAAATCAACATTAGTTTATGCATTTTGAGTTTTTAATAATCATGTTTGCTACTGATTTTCATG
AAGTTGATGGCTCTTCTTTTCCTTTTGCTATTGGAATGGCTAGAAATAGTTTGTTTATTTTTCAT
TTGATTTCAGAAGGGTTATTTCCTAGTAATGTAACAGGCAAAGTTTGTTTAA

TIP sequences:

>NM_001393788.1 Cucumis melo aquaporin TIP1-1 (TIP1-1), mRNA
AGGCGGAGAATCGGATTCGTCATATCAGCAAAAGAGAAAAGTATCACTTCCACTCTTAGAACT
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GCCGCCCTGAAGGCCGCTTTGGCCGAGTTTATTTCTACTCTTATCTTCGTTTTTGCCGGCCAAG
GTTCCGGTCTGGCTTTCTCTAAGCTCACTCATAATTCACCCACCACTCCGGCTGGCCTCATCAT
CGCCTCCATAGCTCATGGCTTTGCCCTTTTTGTCGGTGTCTCCACCGCCGCAAACATCTCCGGT
GGACACCTTAACCCCGCCGTGACTTTCGGGGCCTTGCTCGGCGGTAACATCACTATCCTTCGCG
GTATTCTTTATTGGATCGCTCAGCTCCTCGGCGCCGTCGTCGCTAACTTGTTGCTCAAGTTCGT
CATCGTCGACGTGGCAATTACGGGATTCTTGCCAACAGCAGGGGTTGGAATATGGGAAGCATT
TGTATTCGAGATTGTAATGACATTCGGTTTAGTCTACACTGTCTACGCCACCGCCATTGATCCC
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TCTCTTGGTCTTGGGTTAACCATTGGATCTACTGGGCTGGCCCTCTCATCGGCGGCGGCTTAGC
CGGCATTGTGTATGAACTGTTCTTCATTGGCTTCACCCACGAGCCTCTTCCGACTGCAGAGTAC
TGAAGAAGTGTATTATTGATCTGTTGTAGTCATTAACCTTTCTTCTCTTCGTCAACTTCTTTGTT

CTACGTTTCTCTTCATTAATCTGTCTTCCTCTTCAACTTTTCAATGTCATCCATTTTGATGTCGTA
TTAAATTGATGTCTTCTTCCCCCCTTGAA

>NM_001297726.1 Cucumis melo aquaporin TIP1-2 (LOC103500838), mRNA
ACATGGGGGAGTACTACAAACTACAATTAATTTTAAAGAATTTTAATTTTAAAGTTCTCTAATT
ACTATTAGCCATGCCGATCCGTAACATTGCAATTGGAAGGCCCGAAGAGGCTACTCATCCAGA
TGCTTTGAAGGCTGGATTGGCCGAGTTTATTTCCACCTTGATTTTTGTTTTTGCTGGCCAAGGTT
CCGGCATAGCCTTTAGTAAGCTCACCAATGACGGCGCTGCCACTCCAGCCGGTCTCATCTCCG
CCTCCATCGCCCATGCCTTTGCTCTTTTCGTTGCCGTCTCCGTTGGCGCTAACATCTCTGGTGGC
CATGTTAACCCCGCCGTTACCTTTGGTGCCTTCGTTGGCGGTAACATCACCCTCCTACGTGGTA
TCATCTACTGGATTGCTCAACTCCTCGGATCCGTTGTCGCTTGCTTGCTCCTTAAATTCGTCACC
GGCTTGCCCACCGGAAGCTTCGGTCTATCAGCAGGAGTTGGTGAACTAAACGCCTTCGTTTTC
GAGATCGTGATGACATTCGGCTTGGTGTACACGGTGTACGCAACCGCAGTGGATCCCAAGAAG
GGCAGTTTGGGAACAATTGCACCCATCGCAATCGGTTTCATCGTCGGAGCCAACATTTTGGCC
GGCGGAGCATTCACCGGAGCCTCCATGAACCCCGCCGTGGCATTCGGCCCATCGGTCGTCAGC
TGGTCATGGGAAAGCCACTGGGTCTACTGGGCCGGACCTTTGATCGGTGGTGGCCTTGCTGGT
CTCATCTACGAATTCATCTTCATTTCCAACTCCCACGAGCAACTCCCAACCACCGACTACTAAG
TCAACCACCACCACCACCACCCACGGCGGATGCGGCGGCGACGGCGGCCACTTCTTTTCTTTT
CTTTTTAATCTGGTGTATTTGGTTTTGTTAAGTTAATTTCTTTTATCGGATGGGTTTGGTCGTGT
CTCAAAAAAAAAAAAAAAA

>XM_008465271.2 PREDICTED: Cucumis melo aquaporin TIP1-3-like (LOC103501648), transcript variant X1, mRNA
AAAAACAAAAGTAGAGTGACATAGAAAAACTGTTCTTCAATCTCTTAGAACTGAAAATAAAA
GGAAGAAAAATGCCAATCCATAAGTTTACATTCGGATCACCGGGAGAGGCGAGCCAACCGGA
CGCGATAAAGGCATCATTCGCCGAATTCTTCTCCATGATCATCTTTATTTTCGCCGGACAAGGA
TCCGGATTAGCTTTTGATAAATTAACGGACGGTGGATCTACGACAGCGTCGGGGCTGATAATG
GCATCTCTGGCTCATGCTTTTGCGTTATTTGTGGCGGTTTCGGTGGGAGCGAATATCTCCGGCG
GGCATGTGAATCCGGCAGTTACATTTGGTGCCCTTGTTGGTGGAAACATATCGTTTTTTAGAAG
TATAATGTATTGGATTGCTCAATTGCTTGGCTCTGTTGTTGCGTGCTTCCTTCTTAAGTTTGCCA
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AGAGGGAGAATTTAGGTATCATTGCCCCGATTTCAATTGGTTTCATTGTTGGTGCTAACATTTT
AGTTGGTGGTGCTTTTGATGGTGCTTCCATGAACCCTGCTGTCACCTTTGGCCCTGCTGTTGTC
ACTTGGTCATGGACCCATCACTGGGTCTACTGGCTAGGTCCTATGACTGGTGCAACCATTGCTG
CCGTTGTTTATGATACACTCTTTATCTCTGACTCCATGCATGATCCTCTTACTCAGTATAATGAT
TTCTAGAACCATTTATTCCCTCTAAAACTAGAACAAAGATTTGAAGTTTCTGCTGCAATTTTTG
GACTCATGTTTTTGATCAAGAGTGATATTTTTTTTTAAATGTTTTGTTTTTCTTGACTTTATTCAG
AGACCGAATAGATTCTTTTTATTTATATTGTATGGGTTGATTCCACATTGAAGATTTTGATTGA
CAGAGCTAAGAATCAGGAAGAGAATATATGGATATAGCTTATTATTTTGTTATTAAAGACATT
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>XM_008463955.2 PREDICTED: Cucumis melo aquaporin TIP2-1 (LOC103500601), mRNA
ACTAAGTAGGATTGTGAGCCACATCACAAGTAAAGATATATCATAGGAGACATATTAAAAAG
GAAGTTTGGGGACATGTTCACATGGGTATATCAGAAAAATGCCCTGAATGACAGAAAAGAAT
GAGTGATGATATTAGCTTTAATTGTAATTGTTTGTAATGTCTATATAAGAAGAACAAGAAGAA
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TCACCATTGGAAGCTTTCAAGATTCCTTGAGCTTGCGCTCTTTTAAGGCTTATCTTGCTGAGTT
CATCTCCACCTTACTCTTCGTTTTCGCCGGTGTCGGCTCCGCCATTGCATATAATAAAATAACA
TCATCTGGAGCTTTAGATCCAGCAGGGCTGGTTGGGGTGGCAGTTTGCCATGGATTTGCTCTGT
TTGTTGCGGTCTCCATCGGAGCTAACATCTCCGGTGGCCACGTGAACCCAGCGGTGACATTCG
GGCTTCTTCTTGGAGATCAAATCAGCCTAATTACCACCATTTTCTACTGGATTGCTCAGCTTCT
TGGCTCCATTGTCGCCTGCTACCTTCTCAAATATGTCACTGGCGGCCTCGCGGTTCCGGTCCAC
AGCGTCGCCGCCGGCATCGGAGCGGCGGAAGGGGTAGTGACGGAGATTGTCACCACCTTCGG
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GCCGATTGCCATTGGTTTAATCGTCGGAGCAAACATCTTGGCAGCCGGACCGTTTTCCGGTGG
ATCGATGAACCCGGCCCGATCTTTCGGCCCGGCGGTTGTCAGTGGCGACTTTCACGATAACTG
GATTTACTGGGTTGGGCCGCTCGTTGGCGGTGGCTTAGCTGGGCTTATCTACTTTTACGCCTTT
ATGGCTTATGGGCCCAGCCCAATTCCAAACGATTTCTAAGGCCCAATCAAATTAAACCCATTT

TATTCTCCTTTTGCTCTGTTCCCTTTGTAATAAGAGGAGGAAAAGCAAGTTTTTTTGCCTTTCTT
CCTTTCCATTTTCTCCCCTTTTGGTGTTTTGTTTTAATGTCTTTAATTTATTTGAATGCATGGTTT
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ATTATCTATTAATTCTATCAACCAAA

>XM_008438844.1 PREDICTED: Cucumis melo probable aquaporin TIP2-2 (LOC103482603), mRNA
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TGCTTTGGTATTATATAAACCCCATAACTAAGCTGCTCTCAGACCATCCCTCAGCTCCGGCGAG
AATCTTCTACCTTCTTCTCTCTATTTACTCAACTCTTTCCTTCTTCGGTTTTCCCTCAACGAAAAT
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TCTGAGTTCATCGCCACTCTCCTTTTCGTTTTCGCCGGTGTTGGCTCCGCCATTGCCTACGGTAA
ATTGACATCCGATGCAGCATTGGATCCACCCGGTTTGGTGGCAGTGGCGGTGGCTCATGCATT
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ACCTTCGGTTTGGTCTACACGGTTTACGCCACCGCCGCAGACCCCAAGAAGGGCTCTCTTGGA
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TTTTCTCCCTTTTTATTTCAATGTTTTGCTTTTGAGGTATTTTCCTTTTGTCAATGTACTTTTCTTT
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>NM_001393789.1 Cucumis melo aquaporin TIP3-1 (TIP3-1), mRNA
TGATTCTGTTTTTCACCGCCGTTATGCCGCCGAGACGATACGCTTTTGGACGGGCCGATGAGGC
CACCCACCCCGACTCCATTCGTGCCACCTTGGCTGAGTTCATTTCCACTTTCATCTTCGTCTTCG
CTGGCGAAGGCTCCGTTCTCGCTCTTGATAAAATTTTCAGGCCAGCGGATTATGGAAGTTACG
GCCATGGAAGTTACGGCCGAGGAGGTCACGGTTATGGTCACGGATATGGTAGAAAGGGGGCC
GACACAGGGAGAGCTGCGTCGGATTTAGTAGTGATAGCGATAGCACACGCGTTTGCACTGTTC
TCGGCGGTGGCAGCGAGCATCAACATATCGGGTGGGCATGTGAATCCTGCAGTAACGTTTGGG
GCCCTTATTGGAGGGAGGATCTCTCTTATCCGTGCATTCTTCTATTGGGTTGCACAGATTTTGG
GTGCCATTATCGCTTCACTTCTCTTGAGACTTGCAACGGGTGGCATGAGGCCTATGGGTTTCTT
TGTTTCATCAGGCGTATCAGAATTGCACGGGTTCCTACTAGAGATAATCCTTACATTTGCTTTG
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ACTGGCAATCGGGTTAATAGTCGGGGCCAATATTCTGGTGGGTGGGGCCTTCGATGGGGCCTG
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TTCTGCTCTATATGTTGTAGGGTTTTTTTTTCTCTTTGAAAAAGACTCGAAGCCTTTTATTGGCC
AAGGGTTGTTTGTTGTCTCCGTTTGGGGTTTATTTGTGGTGTTTATGTTCATGAAGGTAATAAA
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>XM_008446797.2 PREDICTED: Cucumis melo aquaporin TIP4-1 (LOC103488186), mRNA
AAATATAGTTCAAGTCAACGAACACAAATGTAATTTACTTACGTGGCACAATAATATGTCATC
ATACTTACCATTTGCCCACATGATGAATTGCTTATAAAACCGCACTTAGTTTTATTCAGAAAAT
TGCCAACCAAACTCCTTTCCAAGTTTCTTGTAGTTCAGTCCTTGAACTCGCCAGAACCAATGGC
CAAAATTGCAATTGGAAGCATCGGCGAGGCCTACCAGCCCGATTGCATCCGAGCCCTCATCGT
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TTATTGGCAAACGCACTTGTCGGTTTATTCGCCGTTGCAGTTGCTCATGCCTTTGTTGTGGCTGT
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TCGGTGGCCACATCACAGTCGTTCGATCCGCTCTATATTGGATTGTTCAGTTGCTAGCAGCTTC
AGCTGCCAGCTTCTTGTTAACGTACCTCACCGGAGGCTTGGTCACTCCAATTCATACGTTAGCA
AGTGGGGTTGGGTATCTTCAGGGAGTGATATGGGAGATTATTCTGACATTCTCCTTGCTTTTCA
CTGTGTATGGTACAATTGTTGACCCAAAAAAGGGGGCTCTTGATGGGCTGGGTCCATTGCTGA
CTGGGTTTGTGGTGGGGGCCAACATCTTGGCTGGTGGAGCTTTTTCAGGAGCTTCAATGAACC
CAGCAAGATCATTTGGGCCTGCTTTGGTGGCTGGAGACTGGACTGACCATTGGGTTTACTGGG
TTGGGCCTCTTATTGGTGGTGGGCTTGCTGGATTCATCTATGAAAACTTCCTCATTCAAAGATC

TCATGTCCCTCTACCTAGGGAGGAAGATGGCTATTAGATTTCATTTTTAAGTCTGTTAAGTTTA
AGCTTCAGCTGATGCAGTTTGTGTTTATGAATTAGAACTTTTGGTTGGATGTAGTGGAAGAGTG
GGGGTGGGTTGTTAAGTGTAATTTTGTTCTTAACTAATAAAAAGTTTGTTAATAGTTTATGAGC
GA

>XM_008469087.2 PREDICTED: Cucumis melo probable aquaporin TIP5-1 (LOC103504693), mRNA
ACTTCAATTTCTCCTTCCATTTCCTTCTTCAATGTTCTTCTGCAAAATGGCCCCTACTTCCCTCG
CTTTCCGCTGCCGACAGTCCATCACCCCAACTGCAATCCGATCCTACGTCGCCGAGTTCATCTC
CACCTTCTTCTACGTCTTCTCTGTCGTTGGAGCATCCATGGCCTCCCAGAAGTATATGCCAGGC
ATTACTACGACAGACCTATCGAGTCTTCTGGTGGCCGCTATTGCCAATGCCTTTGCTTTGGCTT
CAGCCGTATACATCGCCGCCAACATTTCCGGTGGACACGTCAACCCGGCAGTGACGTTCGGAA
TGGCCATTGGTGGCCATGTCAGCGTTCCCACTGCTCTCTTTTACTGGTTTGCTCAAATGCTTGC
CTCTGTCATGGCCTGCATTATTCTTAGAGCCACCATTGTTGGACAGCGCGTTCCAAGCTATGCC
ATTGCCGATGAGATGACGGGGTTCGGAGCGTCGGTGGTGGAAGGTGTATTGACATTTGCACTA
GTATACACCGTTTTTGCCGCCAGTGATCGGAGACGAGGTCCATGCAATGCGATTGGCGCGGTG
ATGATTGGGCTGATTGCTGGAGCCAATGTGCTGGCTGCGGGGCCATTTTCGGGTGGATCGGTG
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TGGGTTGGGCCATTGATTGGGGCGGCTTTGGCTGGAATTGTTCATGATAATGTGGTTTTTCCGG
CTGAAAATGTAGATTCGTTCAGAGGGGTTCCTGAGGCTGTTATCGCTTGATTTTTTAAGCGTTG
GTTTTGTGCAAAAATTGAATGTTTTTGCATTGGTTGTTGTAGTTTATTATACGTATAATTTGTGT
ATCTAACATGAATTTTTATCTTTATTTATTTGTTGTATTTGTTGTTTTGTTTTAATTAATTAAG

NIP sequences:

>XM_008441485.2 PREDICTED: Cucumis melo aquaporin NIP1-1 (LOC103484424), mRNA
TTACAAAACAAATATACATTGGTCAATCCTCTCTTTTTCTCTCTTTTCATGATTTCACCCTTTTT
GGGTCTCTATAAATTCCATCTATTCTTCTGGGTTTTGGCACAATTCTGTGAAACAAACACAAAT
AAGTCTAACCAATCTCAAGAATCCTACACTGTCTTTGCTCAACTCTGTTTGCTTTTTCCTTCACT
CAATCATATTCATGGCTGAGATTTCAGGATCAAGCAATGGACATCATTCTGTTTCTTTGAACAT
CAAAGATGAATCCACCGCCATCACCAGCAGAGAAGTAGCAGCTGAATGGGTTTCTGTATCTTT
CATTCAAAAGTTGATTGCTGAGATTGTGGGGACATATTTCTTGATTTTTGCTGGTGGGGCATCA
GTGGTTGTGAATTTGAGCAAAGACAAAGTCATCACTTTCCCAGGGATTTCAATTGTTTGGGGTT
TGGTTGTAATGGTGATGGTTTATTCTGTTGGTCATATATCTGGTGCTCATTTCAACCCTGCTGTT
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CAAGTTCTTGGATCAACATTGGCAGCTGGGACACTTAGGCTAATATTTAATGGACACGAAGAT
CACTTTTCAGGGACACTCCCAAGTGATTCATATTTGCAAACCTTTGTGATTGAATTCATCATCA
CATTTTATCTCATGTTTGTAGTGTCTGGTGTTGCCACTGACAACAGAGCTATTGGTGAACTTGC
TGGACTTGCTGTTGGTGCTACTGTTCTTCTCAACGTTATGTTTGCAGGGCCAATTACAGGAGCA
TCCATGAATCCAGCCAGAAGTTTGGGACCTGCTATAGTATCAAGGCAATTCAAAGGGTTATGG
ATATACATTGTAGCTCCCATTTTTGGTGCAATTACAGGTGCTTTGGTTTACAATACAATCAGGT
TCACTGACAAGCCTCTACGAGAGATCACTAAAAGTGCTTCTTTTCTCAAAGGACAAAGTCGCA
GAGGTTCATCTTGAGGAGCTTTTGTCTTTCTCACTTTCTAATTCATAAAGATGAAATTTTTCTAA
GTGGGGTTATAGCAAATTAAGAGCATTTTTCTTTTTGTTTTGTATTGCATAGTTAAAGAAGATG
GCATTACTCAAATTGTTATAGTTTTAGTCTTAGTTTATCCAAAAAACATGGACACGAACAACGT
CCTTACTTCACATGAAACTAGGTTAATGTCGGGAATCACGAGTAAAACTAAGAATAAACTACA
TATGGATAAGGATAAGTCCAAACTTCTTGATGGCTGCCTGCTAGTTCA

>XM_008445189.2 PREDICTED: Cucumis melo aquaporin NIP2-1-like (LOC103487002), mRNA
GTAGCTTGCACCTTCAACTTATATGCAAAGAAGTGGTTTTTTCTTTATAATAATATTAGCGTTT
ACGTTCAAATAGAGGGAGATCAATACTCTCATTGTTATTTCTCCCTCTTTCTCATTCGTAAATC
TATAATTTGCCAAAGCTATTATAGAGTTCTAAAACCACTGTTTTAAAGAACTGCTTTTTCAGTA
CTTCTTAATTTCTGTTTCTTCCCAATACAAGGAAACAAAAATGAGTTCTATAAATCCTGAGCTC
TCCAACCAAGAAACTGTTGTGGATGTTAATGAATTCGTATCTGTTGAAAACCCAGATTCTAAA
CGCTCCAAGTTTGGATCTTTCTTCAAAAACCCTTACCCTCCTGGGTTTTCCCGAAAGCTCGTGG
CGGAGGTGATAGCAACCTATTTGCTAGTGTTTGTGACATGTGGGGCGGCAGCATTGAACGCGA
GCGATCCACAGAGAGTGTCGCAGCTCGGTGCTTCAGTTGCTGGTGGATTGATCGTGACTGTGA
TGATTTACGCGGTCGGACACGTTTCCGGTGCCCATATGAACCCGGCTGTCACCATGGCTTTTGC
TGCAACTCGACACTTTCCATGGAAACAGGTTCCATTGTATGGAGCAGCACAATTGAGTGGAGC
AACTTGTGCAGCATTTACATTGCGCTTATTATTGCATCCAATTAAGCATTTGGGCACAACTACA

CCATCAGGATCTGATTTACAAGCTTTAGTTATGGAGATTGTTGTTACATTCTCTATGATGTTTGT
CACTCTTGCTGTTGCAACTGACACCAAAGCAGTAGGGGAGCTGGCAGGTATGGCAGTTGGCTC
AGCTGTTTGTATCACTTCCATCTTAGCTGGACCTGTATCAGGAGGGTCAATGAACCCTGTGAG
GACTTTAGGACCTGCATTGGCAAGTGATAATTATAAAGGGCTTTGGGTATACTTTGTTGGGCC
AGTTGTTGGAACTCAATTGGGGGCATGGTCATATAAGTTCATACGTGCCAGTGACAAACCTGT
GCACTTAATTTCTCCCCATTCTTTTTCACTCAAACTTCGAAGGATGTCGAGATCAGATGTTAGC
GAAAGCAATCACTGATATGAGCATTGTCTTTCGCATCGTCTCCCATCTTTAAAATAATTGAGAG
ATTTAAAATGATGGAAAGATGTGAAATTAATAGTTTGAGTGTTTTAATGAGCAATGATCTCAT
GTATACTATGCTATAAGCTTCTTTTATCTTATAGTTTAGCAATGTATGAGGGTAACTTTTTTAGA
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AAGATTTTCTACTTTTTGTAACA

>NM_001393790.1 Cucumis melo aquaporin NIP2-2 (NIP2-2), mRNA
CATCCACACTACGACTAACACTAACACCAATTACTTTCGTTGCCTATAAGATATTTCCGTCCCT
TCCCTTCCCTTCCTTCAAAAATCAAAACTAATTATTCAAACATATATTAAATCGCTCCCAATTT
TTCAAATTCCCTAAAGAAATGGCGAGACGCAACGACAATGAAGAAGCCTTTATTGCCTTGGGA
AACGAATGCTCTGATCCTCAACCATCCTTGTTCCGCGACCGATTTGATGAAGTTTACCCACCCG
GGTTCTCACGAAAGCTTGTGGCGGAGGTGATTGCTACGTATTTGCTTGTGTTTGTGTCGTGTGG
TGTGGCGGCATTGAGTGGGAGTGATGAACAGGAAGTGTCGAAGCTTGGAGCTTCAATTACCTG
TGGATTGATTGTCACGGTGATGATTTACGCCGTCGGACATATCTCCGGCGCTCATATGAATCCA
GCGGTTACTATTGCTTTTGCTGCCGTTCGGCGATTTCCATGGAAACAGGTTCCACTTTACGCAG
CAGCTCAACTAAGTGGAGCTACATCGGCAGCCTTTACACTACGCATATTATTGGATCCAATTC
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CGGAGGCATAGCTGTGGGGTCGGCTGTATGTATCTCCTCCATCTTTGCTGGGCCAATATCGGGT
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TTGGGTGTATATGATCGGTCCAATTACAGGAACTTTGCTTGCGGCATTTTCATATAATTTCATA
CGAGCCACTGAAAAACACACCCATTCACTCTCGTCACATTGACATCCAATCAATCGGATCGTG
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ATTGTCTTGCAATTTATTTGTTTTTTTAGTTTTGAGAGAAGAATGAATCTGAAATAGGGAATCA
TTTTTATTATTATTGTTATTTTACAAGTGTGCGTTTTGATCTGATTTTTTTTACAATAATCAACC
ATAAAATTGGTTCTTCTTTTCA

>NM_001393791.1 Cucumis melo aquaporin NIP4-1 (NIP4-1), mRNA
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GGAATTGAAGACGAAGAAATTTCAAAGCTCGAAGAAGGCACTGTCGTTTCCGCCATTGCTCGC
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TTCGTGATCTTCAGCGGATGTGGGGCGGTGGCGGTGAACAAAATATACGGATCAGTGACATTT
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CAGGTGCCAATATACACAGGAGCTCAACTAATGGGGTCCCTTCTCGCAAGCTGCACATTGGAT
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TCTTTGGTTCTTGAAATTATCATCACATTTCTTTTGATGTTTGTCATCTCTGGTGTCTCCACCGA
TAATAGAGCCGTAGGAGAATTGGGTGGAATTGTGGTTGGAATGACCATTCTCTTGAATGTTTT
CGTCGCCGGGCCGATTTCTGGAGCTTCTATGAATCCGGCAAGGACTATTGGACCAGCCATAGT
GAAACGGCAGTTTAAAGGACTATGGGTTTACATAGTAGGACCCTTTATCGGAGCGGTCGCCGG
TGGATTTGTCTATAACCTAATGAGATACACAGACAAGCCGCTGCGGGAGATCACCAGAAGCA
CTTCATTCCTCACCGGCACCCTGAAATCATAACCGCCGGCCTCACTCTTCACCACCACAATATG
AACTAGTAGTACACTGTTCAATCATTCATTTATGTATATAGTTTGTTGTCTCCATCAATTGTCAT
TCTTTTA

>XM_008439275.2 PREDICTED: Cucumis melo probable aquaporin NIP5-1 (LOC103482897), mRNA
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ACTCTGGATTTTCTCGTTCAACCCATCTTTCTTAGGTTCTAAGTTAAATTAAGTGTGGCTTCCTT
CTCTTCAACAAATCTTCCTTAACTTCACATTTATAAAAGCTTCCAAATCATGTAACTTTAGCAT
CTTTCTTAATCACCCCCTTCAAAAAAAATCCAAAAAAAAAAACATTTTTTAAATCTTAGATGAAT
TTCTTTTTTATCCCAAACTAAAAATCTAGTGTTTAATATCCTGGCTAAGCTTCCAATTCTGCCTT
AGAACAACTTTTATAAAAATCTCCAAATCATGTAACTTTGTTTTCTTAAATCTCCATCATTCCA

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TTGTGGGGACATTCATCTTGATATTTGCTGCGACGGCAGGGCCCATTGTGAACCAAAAGTACA
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TGTCGACGGGACACATTTCCGGTGCACACCTGAACCCATCACTCACCATTGCCTTTGCTGCCCT
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GCCTCCTTCGCTCTCAAAGGGGTTTTTCACCCTTTCATGTCTGGTGGTGTCACCGTCCCCTCCGT
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TTAGCACA

>NM_001393792.1 Cucumis melo aquaporin NIP5-2 (NIP5-2), mRNA
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AAGCCAATGGCACAATTTGAGATTGTACGCGTGGACTCCCCCAAGCGCAGCCAAATTCTTGAT
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GTAAAGCACAAAGATGATGGAATTGATGTTCTGCCGGAGGAGAGGAGTTTCCATCAGTGATA
ATGATAGTGGTCGTTGCTATATGTTTCTGTTTTAATGTCCAGAGTACACGACCTTTTACTTTCAT
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>XM_008455944.2 PREDICTED: Cucumis melo aquaporin NIP6-1 (LOC103494651), mRNA
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GGAGGCTTATCTAAAATAGTCTCTTCTTGTTCATTGCCATACCCTCCTGTTTCACTTGCAAGAA
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AATCCAGTAAGAACACTGGGGCCAGCCATTGCAGCAAACAATTTCAAAGCCATATGGATTTAC
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GACAAAGATGGTGATTCTCGCTTGCCTTCTACTGCAGCAAGCTTTCGACGATGACAACAGATC

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GGGATCAAACACACTAGAGTCCCTATCAACTCCTCTAAAAACTCTATTATTCTTTCCCTAGAGA
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AGCATGTTAACACAGATAAGCAAGTGCGTCGACATTGGTCGAAGGAAGGACGACAACACACA
AGCAAGGATGTTAATAAAGAGCCTGGGGATATCATTATTACTCAAAATTGGAAGGAAAGATT
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CCTCCTTTCTTAA

>XM_008440492.2 PREDICTED: Cucumis melo probable aquaporin NIP7-1 (LOC103483738), mRNA
GGTTGGCTACACATCAAAATAACTTTACCAAATCACCACAATTTTAACTACAAAAAAATACAC
ACGCCAACTTTAGAATCACATATCATCAAACTCAGGCTTTTTATACTTTCAAACAACCCTTCAA
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TGTCTCCGCCACCTTCCCACGACCGACGACAATGGACGATAATCTTGTTCGCCCGGTTTTGGGA
GAGATGGTGGGGAGTTTTTTGTTGATTGTTTGCGTGAGCGGGGTAACGGCGACCGGGCAGCTG
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CTTCATAATTATTCTTCTCCCTTTTCTGCCTTTTTCCTCGAACTTCTTTTAACTTTCATTCTCATG
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ATGGCCATTGCCCTTGCTGTGTTTATTGCCGGGCCCATTTCCGGTGCATCAATGAACCCAGCAA
GATCTTTAGGGCCGGCCATTGTTTCATGGGCTTTCGATGACATATGGATCTATATTACGGCTCC
GTGCATCGGAGCCATTACCGGCGCCTTCATTAGCGACTTCCTCCGCCTTTGTCCTCCACCGCTT
CAACCTTCCGACGGCAAACATTTTGATCTCTCCTCCTCTGCCAACGCATATCTAATTACTTAGC
AAATAATCGCCCTTCATCATCCCCTCTATATTTCCAGTTGCGTTTTTTTATAGATCCAAATGATC
TATCATCTTATATAGACAGACGTGGAATAACAATTTAGTCATAAAATCGTGTAATTTTATTAAC
CGGTAATCAAATTAAACCTTACTTAGTTGTCTCG

SIP sequences:

>XM_008443748.2 PREDICTED: Cucumis melo aquaporin SIP1-1-like (LOC103485971), mRNA
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TCATCTTTTCCCATTTTTTTTCTCTCACAATTTTCTCTTCTTATTCAACTTCCATTGATTTCGATT
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TTCTCACTTCCATGTGGATTTTCTGTGCTTCATCTCTTGGGGTTCTCACTTCCGTTCTCTACTCA
GCCGCCGGTGTTTATGGAATCCCACTTCACCCACTTCTTATCACTACTACTCTTGTTTTCATTCT
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CAGGCGGCTGGTGCGGTTGCTGGTGCCATGGCAATCAAGGAGGTGATGCCTATACAATACAA
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GACCTTCCATGAATCCTGCAAATGCATTTGGATGGGCATATCTAAACAACAGGCATGATACAT
GGGAGCAGTTGTATGTGTACTGGATCTCCCCCTTCGTAGGAGCCATTTTGGCTGCTTGGCTTTT
CAGAATCATCTTCCCACCACCACCCCCAGCCCCAGCCAAGCAGAAGAAAGCTTAAAACCGGT
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TGTTTAGGCAGTTATGTTTAGTTTGATTCTTATAATTATTTTCTTCAGGCTTGTTTGGATTATGA
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CAAA

>XM_008444982.1 PREDICTED: Cucumis melo probable aquaporin SIP2-1 (LOC103486855), mRNA
CAAGAATTCCTCTTAATGATTCCAATGTAGTGTAAAAACATTGGGGATGTGTCAATGTCCAAT
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CATACATTACTACGGTAATCTTTGTCCAATCAAAATCAAAGCCACCAATATTCCTTCATTTACC
CTTCTCCTCACTCTTCATCGCTTCAAATTGATCAATCCGAGTGAAGAAACGGGTCGTAGGGAA
GAACCCCAGAAGATTTCCTTTTCAACGATGTCTTCTGGGGCGGCTCGATTGCTTGTGTTGGATT
TTGCCCTCTCTTTTATGTGGTTATGGTCGGGGGTTTTGGTTAAGATCTTTGTTTTTGGGATATTG
GGGTTCGGAGATGATCTGGTTAGCGAGGTTGTCAAGGCTTCCTTTTCGATTCTCAACATGTTCT
TCTTTGCGTTCCTGGTGAAAATTTCAAATGGGTCTGCTTATAATCCTCTTACCATCTTATCTGCT
GCTTTTTCTGGGGATTTCAGTAGATTTCTTTTCGTTGTTGGTGCCAGAATCCCTGCTCAGGTAAT
GGGAGCTATTACTGCTGTTAGGCTGATTATCCATACATTTCCCGAAGCAGGACGAGGGCCTCG
TTTGACTGTTGGCATCCATCACGGCGCTTTGACGGAAGGATTGTTAACATTTGCCATTGTATCT
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TATCCAAGTTAACTCTTCATATCCTCGGTTCCGACTTAACTGGTGGCTGTATGAACCCCGCATC
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GAAAGAAGAGAAAGTAAATATGAAGAAGAAATCAGAATGAGTAACCTTGCTGAATTGAGTTG
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CATGCAAATGTATAGTTTGGTGGGTAAGGCATCGATCACCATTCAAAATGCCAATTGTTCGAC
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XIP sequence:

>NM_001393793.1 Cucumis melo aquaporin XIP1-1 (XIP1-1), mRNA
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GAAGAAGGAAGAAGCTTTGATGAACAAACTTCTTCTTCTTCCTCTTTTCTCTCAAGGTTTCTAA
TTTACATTGGTGCTCATGAACTTTTCTCACAAGAGATGTGGAAAGCAGCAATGACAGAATTAG
TAGCGACTGCCCTTCTAATATTTTGCTTAACAACTTCCATCGTCTCATGCTTGAATTCACACCA
ATCAGATCCAAAGCTCTTAATCCCATTCGCCGTTTTCATCATCCTCTTCCTCTTCCTCGTCGTCA
CTTTCCCCCTTTCCGGCGGCTTCCTCAGCCCCATCTTCGCCTTCATCGCCGCCCTCCACGGCGTC
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CATGATCAAAGATGCAATGAGTCCCGATGTAGCTGACAAATACTCTCTCGGAGGCTGCACCAT
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ACATTCGTCGTTCTCTACGTCGGAGTCACGGTGGTGCTCGACCAGAAGATGAGCGAGCGGTTT
GGTTTGCCAATGGTGTGTGGGATGATTGCGGCGAGTTCGGCGGTGGCAGTTTTTGTGTCGACG
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GGCCTGTGTGGTTTATTATGGATTTTCGAAGAATTTGCCGAATGGGGTGTTGGTTGGAGCGAA
AGGAGAGATTGGGATTTTGAAGATGGTCGGAGGTCGTTGTTGGCGGCGGCGGCGCCAAAAGT
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TATTT