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DISSERTATION

COMPLEX ECOLOGICAL, PHYSIOLOGICAL AND GENETIC ANALYSIS OF THE RESPONSE OF *CUCURBITA* GENUS REPRESENTATIVES TO SALT STRESS

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ABSTRACT

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Soil salinization is a characteristic feature of many natural ecosystems. It also covers about 20% of the arable land of the planet. Currently, salinization, the territory of which is constantly growing, is one of the powerful factors limiting the sustainable development and modernization of world agriculture. Therefore, studies aimed at elucidating the mechanisms of response and adaptation of plant organisms to salt stress are becoming more and more important. First, they should cover the leading agricultural crops, including members of the *Cucurbitaceae* family, which are widely cultivated in the world for their economic and ecological properties. *Cucurbitaceae* are not halophytes, most of them are sensitive to salt stress and usually show obvious inhibition of growth in conditions where the NaCl content in the soil solution reaches 0.1%. Taking into account the above, the question of clarifying the ecological and physiological aspects of the formation of salt tolerance in representatives of the *Cucurbitaceae* family and the actual genus *Cucurbita* and managing this trait on the basis of the use of both classical and modern scientific and technical means is gaining relevance.

Taking into account the above, the issue of clarifying the ecological-physiological, genetic aspects of the response to salt stress of representatives of the *Cucurbitaceae* family and the actual genus *Cucurbita*, the formation of their salt resistance, becomes relevant and managing this trait on the basis of the use of both classical and modern scientific and technical means.

The aim of the work was determined: to establish the mechanisms of response to salt stress and adaptation to it, which are implemented by representatives of the genus *Cucurbita* at different levels of the organization, as well as eco-physiological aspects

of the formation of salt resistance, quantitative and qualitative characteristics of plants when using grafting technology.

The work was performed on the basis of the application of theoretical (analysis, synthesis, explanation, generalization, a mathematical and statistical) and empirical (experiment, measurement, comparison) research methods. Experimental field and laboratory ecological-physiological and laboratory genetic studies were carried out, chemical, spectrometric, fluorescent, morphometric, and vitality analyses were applied, and among mathematical and statistical calculations, point estimation of statistical series and dispersion analysis were used.

In studies aimed at establishing the effect of salt stress on the morphological characteristics, vitality, and degree of damage to pumpkin plants, as well as on the exchange of organic and inorganic compounds, the flow of leading physiological processes, the following experimental scheme was used: control (water, 0 mmol/L NaCl), low salt stress (60 mmol/L NaCl), high salt stress (120 mmol/L NaCl). Pumpkins of two varieties were used in the study: Yanzhen and Miben.

When studying the eco-physiological interactions that occur between plants when grafting technology is used, pumpkin plants that were subjected to salt stress were grown, followed by their use as rootstocks for watermelon. The study of the interactions formed when using salt-resistant pumpkin rootstocks was supplemented by an assessment of the impact of arbuscular mycorrhiza on plant metabolism and resistance.

According to the results of the study of the effect of salt stress caused by different concentrations of NaCl, the nature of the reaction of morphological features of pumpkin to salt stress was determined. It is shown that against the background of increased salt concentration, the size, and vitality of plants decrease, and a number of negative quality signs appear in them: yellowing of leaves, their twisting, etc. It has been established that changes in the dimensional parameters of plants against the background of salt stress can be an informative indicator in the study of the mechanism of salt resistance and the breeding of salt-resistant varieties of pumpkin.

It is shown that plants respond to an increase in the concentration of a salt solution with a statistically significant increase in the salt damage index and the salt damage

rate. The results of the research proved that the salt damage index, as a morphological index for assessing plant resistance to salt, is a simple, easy, accurate and reliable indicator for directly detecting the degree of resistance of *Cucurbita* genus representatives to salt stress.

It has been proven that salt stress affects indicators and signs related to the course of photosynthesis and water exchange of pumpkin plants, which leads to a decrease in the following values: photosynthesis rate, stomatal size, stomatal conductance, transpiration rate, as well as changes in chlorophyll content, mainly in the direction of its increase. At the same time, the reduction of indicators that determine the amount of water loss are factors in increasing the resistance of plants to salt stress, and the indicators that determine the intensity of photosynthesis are factors in slowing down the synthesis of carbohydrates and inhibiting plant growth. The degree of variation in chlorophyll content in leaves under salt stress was related to plant variety and salt concentration.

An increase in the content of MDA (malondialdehyde) and the permeability of cell membrane structures due to their peroxidation was recorded in pumpkin under salt stress. The relative permeability of the plasma membrane also increased against the background of increasing salt concentration. One of the reasons why NaCl damages the structure of the plasma membrane is the excessive accumulation of Na^+ in cells: it replaces Ca^{2+}, which can stabilize and protect the plasma membrane.

It was shown that the transformations related to the metabolism of carbohydrates and amino acids in pumpkin play an important role in the complex of processes of response and adaptation to salt stress: with increasing salt concentration, the content of proline and soluble sugars increases. Soluble sugar and proline are essential substances that regulate the osmotic potential and water potential of plants under salt stress conditions. After 7-day salt stress caused by exposure to NaCl, the proline content in the leaves of pumpkin seedlings was significantly higher than in the control.

It was established that salt stress affects the absolute and relative indicators of the accumulation of ions of mineral substances, as well as their distribution in plant organs. According to these characteristics, Na^+, K^+, Ca^{2+} and Mg^{2+} ions showed high

individuality, which is also a result and evidence of their specific role both in the aspect of ensuring the reaction and adaptation of pumpkin plants to salt stress.

It has been found out that in response and adaptation to salt stress in pumpkins at all levels of the organization against the background of the stated general trends, varietal features are clearly manifested. In particular, it was established that the Yanzhen mainly accumulates Na^+ in the root system, while the Miben mainly accumulates in the stem. This results in the formation of differences in the varieties and in the accumulation of K^+, Ca^{2+} and Mg^{2+}, as well as differences in the value of the K^+/Na^+ ratio. In general, according to a complex of physiological and morphometric characteristics, Yanzhen shows a higher resistance to salt.

Identification and analysis of the expression of the WRKY gene family in *Cucurbita* genus representatives under salt stress conditions was carried out. Screened 12 WRKY family transcription factors in pumpkin in response to salt stress, and analysed their phylogenetic relationships, spatio-temporal expression patterns, tissue-specific expression characteristics, and transcriptional activities under salt stress in detail.

For to improve and expand research aimed at revealing the genetic aspects of the formation of salt resistance, have developed an efficient transient transformation system for the study of gene function and protein subcellular localization. Using this method, the salt tolerance gene StNHX1 was transiently overexpressed in pumpkin roots and cotyledons.

The interactions that occur between the scion and the salt-resistant pumpkin rootstock when using the technology of grafting in the cultivation of gourds were studied. The influence of salt-resistant rootstocks on the metabolism of rootstocks has been clarified and proved, the feasibility of using grafting technology to increase the resistance of plants to salt stress, increase the yield of crops and obtain high-quality products has been shown. It is shown that the use of arbuscular mycorrhizal fungi in the cultivation of melon crops based on grafting technology leads to a change in plant metabolism, affects the quality of the obtained products and contributes to increasing the resistance of plants to adverse environmental factors.

Scientific novelty is inherent in the research results obtained during the dissertation. For the first time, based on the results of observations during the entire growing season, the influence of salt stress on the complex of quantitative and qualitative morphological features of pumpkin plants was investigated. The features and regularities of the accumulation of ions of mineral substances in the vegetative and generative organs of pumpkins under conditions of salt stress have been established. The physiological and biochemical transformations that occur in pumpkins against the background of salt stress have been comprehensively investigated and clarified. The methodological aspects of assessing the impact of salt stress during the pumpkin ontogenesis were improved, and the method of vitality analysis was used for the first time to assess the vitality of pumpkin plants. An efficient system of temporary transformation was developed to study the function of genes and subcellular localization of protein in pumpkin. The theoretical and practical principles of using salt-resistant pumpkin rootstocks for the cultivation of melon crops have gained further development.

The materials of the dissertation work are used in the educational process of the Department of Ecology and Botany of Sumy National Agrarian University. The research results were reviewed and discussed at 6 conferences of various ranks. 12 scientific works have been published based on the dissertation materials: 3 articles in journals included in the Scopus, Web of Science database, 3 articles in specialized scientific publications of Ukraine, 6 publications in conference materials.

Keywords: *genus Cucurbita, soil, salinization, stress resistance, adaptation, morphometric analysis, vitality structure, plant development, antioxidants, metabolism of organic substances, photosynthesis, chlorophyll content, ions of inorganic substances, grafting of plants, gene expression, rhizosphere, microbial cenosis, vital activity of soil microorganisms.*

АНОТАЦІЯ

Хе Сунтао. Комплексний екологічний, фізіологічний та генетичний аналіз реагування представників роду *Cucurbita* на сольовий стрес. – Кваліфікаційна наукова праця на правах рукопису.

Дисертація на здобуття наукового ступеня доктора філософії за спеціальністю 101 «Екологія». – Сумський національний аграрний університет, м. Суми, 2023.

Засолення ґрунту є характерною ознакою низки природних екосистем. Воно охоплює ще й близько 20% орних земель планети. Натепер, засолення, територія поширення якого постійно зростає, є одним із потужних чинників, що обмежують сталий розвиток і модернізацію світового сільського господарства. Тому усе більшої значущості набувають дослідження, спрямовані на з'ясування механізмів реагування рослинних організмів на сольовий стрес та адаптації до нього. Насамперед вони мають охоплювати провідні сільськогосподарські культури, включаючи представників родини *Cucurbitaceae*, які широко культивуються у світі завдяки своїм господарським та екологічним властивостям. Гарбузові не є галофітами, більшість із них чутливі до сольового стресу і зазвичай вже демонструють пригнічення росту коли вміст NaCl у ґрунтовому розчині досягає 0,1%. Враховуючи зазначене, актуальності набуває питання з'ясування еколого-фізіологічних, генетичних аспектів реагування на сольовий стрес представників родини *Cucurbitaceae* і власне роду *Cucurbita,* формування їхньої солестійкості та управління цією ознакою на основі використання як класичних, так і новітніх науково-технічних засобів.

Метою роботи було визначено: встановити механізми реагування на сольовий стрес та адаптації до нього, які реалізуються представниками роду *Cucurbita* на різних рівнях організації, а також еко-фізіологічні аспекти формування солестійкості, кількісних та якісних характеристик рослин при використанні технології щеплення.

Робота виконана на основі застосування теоретичних (аналіз, синтез, пояснення, узагальнення, математико-статистична оцінка) та емпіричних

(експеримент, вимірювання, порівняння) методів дослідження. Реалізовано експериментальні польові й лабораторні еколого-фізіологічні та лабораторні генетичні дослідження, застосовано хімічний, спектрометричний, флуоресцентний, морфометричний, віталітетний аналізи, а з-поміж математико-статистичних розрахунків використано точкове оцінювання статистичних рядів та дисперсійний аналіз.

У дослідженнях, спрямованих на встановлення впливу сольового стресу на морфологічні характеристики, життєвість (віталітет) і ступінь ураження рослин гарбуза, а також на обмін органічних і неорганічних сполук, протікання провідних фізіологічних процесів, використовували наступну схему досліду: контроль (вода при 0 ммоль/л NaCl), низький сольовий стрес (60 ммоль/л NaCl), високий сольовий стрес (120 ммоль/л NaCl). У дослідженні були задіяні гарбузи двох сортів: Яньчжень і Мібен.

При вивченні екофізіологічних взаємодій, які виникають між рослинами при використанні технології щеплення, проводилось вирощування рослин гарбуза, які зазнали сольового стресу, із наступним їх використанням як підщепи для кавуна. Вивчення взаємодій, які формуються при використанні солестійких підщеп гарбуза, було доповнено оцінкою впливу арбускулярної мікоризи на обмін речовин та стійкість рослин.

За результатами дослідження впливу сольового стресу, викликаного різними концентраціями NaCl, встановлено характер реакції морфологічних ознак гарбуза на сольовий стрес. Доведено, що на тлі підвищення концентрації солей відбувається зменшення розмірів і віталітету рослин та прояв у них низки негативних якісних ознак: пожовтіння листків, їхнє скручування тощо. Показано, що зміни розмірних показників рослин на тлі сольового стресу можуть виступати інформативним індикатором при дослідженні механізму солестійкості та виведенні солестійких сортів гарбуза.

Встановлено, що рослини реагують на підвищення концентрації сольового розчину статистично значущим підвищенням величин індексу сольового ураження та ступеня сольового ураження. Результати досліджень засвідчили, що індекс сольового ураження, як морфологічний індекс оцінки стійкості рослин до

солі, є простим, легким, точним та достовірним показником для прямого виявлення ступеня стійкості представників роду *Cucurbita* до сольового стресу.

Доведено, що сольовий стрес впливає на показники та ознаки, пов'язані з перебігом фотосинтезу та водообміну у рослин гарбуза, що призводить до зниження значень: швидкості фотосинтезу, розміру продихів, продихової провідності, швидкості транспірації, а також до зміни вмісту хлорофілу, переважно у бік його збільшення. При цьому зниження показників, що визначають величину втрат води, є чинниками підвищення стійкості рослин до сольового стресу, а показників, що визначають інтенсивність фотосинтезу, – чинниками уповільнення синтезу вуглеводів і гальмування росту рослин. Ступінь варіації вмісту хлорофілу в листках на тлі сольового стресу був пов'язаний з сортом рослин та концентрацією солі.

У гарбузів за сольового стресу зареєстровано підвищення вмісту МДА (малонового діальдегіду) та проникненості мембранних структур клітин унаслідок їхнього перекисного окислення. Відносна проникненість плазматичної мембрани також зростала на тлі збільшенням концентрації солі. Однією з причин пошкодження NaCl структури плазматичної мембрани є надмірне накопичення Na^+ у клітинах: він замінює Ca^{2+}, який може стабілізувати та захистити плазматичну мембрану.

Показано, що у гарбузів перетворення, пов'язані з обміном вуглеводів та амінокислот, відіграють важливу роль у комплексі процесів відповіді та адаптації до сольового стресу: зі збільшенням концентрації солі збільшується вміст проліну та розчинних цукрів. Вони є важливими речовинами, які регулюють осмотичний потенціал і водний потенціал у рослин в умовах сольового стресу. Після 7-денного сольового стресу, обумовленого впливом NaCl, вміст проліну в листках проростків гарбузів був достовірно вищим, ніж у контролі.

Встановлено, що сольовий стрес впливає на абсолютні та відносні показники накопичення іонів мінеральних речовин, а також їхній розподіл органами рослин. За цими характеристиками іони Na^+, K^+, Ca^{2+} та Mg^{2+} продемонстрували високу індивідуальність, що також є результатом і свідченням

їхньої специфічної ролі як в аспекті забезпечення реагування, так і адаптації рослин гарбуза до сольового стресу.

З’ясовано, що у реагуванні та адаптації до сольового стресу у гарбузів, на усіх рівнях організації, на тлі вищевикладених загальних тенденцій, чітко проявляються сортові особливості. Зокрема, встановлено, що сорт Яньчжень переважно накопичує Na^+ в кореневій системі, а Мібен – у стеблі. Це має наслідком формування відмінностей між сортами й у накопиченні K^+, Ca^{2+} і Mg^{2+}, а також відмінностей у значенні співвідношення K^+/Na^+. Загалом, за комплексом фізіологічних та морфометричних ознак, Яньчжень демонструє вищу стійкість до солі.

Проведено ідентифікацію та аналіз експресії родини генів WRKY у представників роду *Cucurbita* в умовах сольового стресу. У гарбузів при реагуванні на сольовий стрес відкрито 12 транскрипційних факторів родини WRKY, детально проаналізовано їхні філогенетичні зв’язки, просторово-часові моделі експресії, тканино-специфічні характеристики експресії та транскрипційну активність під сольовим стресом. Задля вдосконалення та розширення досліджень, спрямованих на виявлення генетичних аспектів формування солестійкості, розроблено ефективну систему транзиторної трансформації для вивчення функції генів та субклітинної локалізації білків. Використовуючи цей метод, ген стійкості до солі StNHX1 був тимчасово надмірно експресований у коренях і сім’ядолях гарбуза.

Вивчено взаємодію, яка виникає між прищепою та солестійкою підщепою гарбуза при застосуванні технології щеплення при вирощуванні баштанних культур. З’ясовано та проаналізовано вплив солестійких підщеп на метаболізм підщеп. Доведено доцільність використання цієї технології для підвищення стійкості рослин до сольового стресу, підвищення врожайності культур та отримання високоякісної продукції. Показано, що використання арбускулярних мікоризних грибів, при вирощуванні баштанних культур на основі технології щеплення, призводить до зміни метаболізму рослин, впливає на якість одержуваної продукції та сприяє підвищенню стійкості рослин до несприятливих чинників довкілля.

Результатам досліджень, отриманим у процесі виконання дисертації, притаманна наукова новизна. Зокрема, уперше, за результатами спостережень протягом усього вегетаційного періоду, досліджено вплив сольового стресу на комплекс кількісних та якісних морфоознак рослин гарбуза. Встановлені особливості поглинання та накопичення іонів у вегетативних та генеративних органах гарбузів за умов сольового стресу. Всебічно досліджено та з'ясовано фізіолого-біохімічні перетворення, які відбуваються в гарбузах на тлі сольового стресу. Удосконалено методичні аспекти оцінки впливу сольового стресу протягом онтогенезу гарбуза та вперше використано методику віталітетного аналізу для оцінки життєвості рослин гарбуза. Розроблено ефективну систему тимчасової трансформації для вивчення функції генів і субклітинної локалізації білка в гарбузах. Набули подальшого розвитку теоретичні та практичні засади використання солестійких підщеп гарбузів при вирощуванні баштанних культур.

Матеріали дисертаційної роботи використовуються в навчальному процесі кафедри екології та ботаніки Сумського національного аграрного університету. Результати досліджень були розглянуті та обговорені на 6 конференціях різного рангу. За матеріалами дисертації опубліковано 12 наукових праць: 3 статті у журналах, що входять до баз даних Scopus, Web of Science, 3 статті – у фахових наукових виданнях України, 6 публікацій – у матеріалах конференцій.

Ключові слова: рід *Cucurbita*, *грунт, засолення, стійкість до стресу, адаптація, морфометричний аналіз, віталітетна структура, розвиток рослин, антиоксиданти, обмін органічних речовин, фотосинтез, вміст хлорофілу, іони неорганічних речовин, щеплення рослин, експресія генів, ризосфера, мікробний ценоз, життєдіяльність грунтових мікроорганізмів.*

LIST OF PUBLISHED WORKS ON THE THEME OF THE DISSERTATION

Publications in journals on scientometric bases Scopus, Web of Science:

Yang, P.M., **He, S.T.,** Jiang, L.N., Chen, X.J., Li, Y.F. & Zhou J.G. (2020). The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose content of different ploidy watermelon (*Citrullus lanatus*). Photosynthetica. 58 (5), 1150–1159. DOI: 10.32615/ps.2020.068

Xuejin Chena, **Songtao He**, Lina Jiang, Xinzheng Li, Weili Guo, Bihua Chena, Junguo Zhoua & Viktoriia Skliar (2021). An efficient transient transformation system for gene function studies in pumpkin (*Cucurbita moschata* D.). Scientia Horticulturae, 1, 1–10. DOI:10.1016/j.scienta.2021.110028

Yang, P.M. & **He, S.T.** (2022). The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*). Hort. Sci. (Prague), 49, 225–233. https://doi.org/10.17221/108/2021-HORTSCI

Articles in professional publications of Ukraine:

Шерстюк, М.Ю., Скляр, В.Г., Скляр, Ю.Л. & **Хе Сунтао** (2019). Комплексний популяційний аналіз як напрямок сучасних біолого-екологічних досліджень. Вісник Сумського національного аграрного університету. Серія «Агрономія і біологія», 3 (37), 61–67. DOI: https://doi.org/10.32845/agrobio.2019.3.10

Хе Сунтао (2022). Фізіолого-біохімічні аспекти реагування рослин на засолення ґрунту (оглядова). Вісник Сумського національного аграрного університету. Серія: Агрономія і біологія, 50 (4), 62–68. https://doi.org/10.32845/agrobio.2022.4.9

Хе Сунтао (2023). Щеплення у системі заходів із вирощування рослин родини *Cucurbitaceae*. Вісник Сумського національного аграрного університету. Серія «Агрономія і біологія», 51 (1), 129–136. DOI https://doi.org/10.32782/agrobio.2023.1.15

Abstracts of scientific reports:

He Songtao, Skliar V.G. & Zhou Junguo (2019). Effects of different concentrations of salt on pumpkin seedlings. Матеріали науково-практичної конференції викладачів, аспірантів та студентів Сумського НАУ (м. Суми, 17–20 квітня 2019 р.), 29.

He Songtao, Zhou Junguo & Skliar V.G. (2019). The problem of soil salinization and the role of genetic engineering in increasing the salt tolerance of plants. Матеріали Міжнародної науково-практичної конференції, присвяченої 90-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (м. Суми, 24–25 травня 2019 р.), 13.

He Songtao, Skliar V.G., Zhou Junguo & Xinxiang (2020). Effects of salt stress on the resistance of vegetable cytoplasmic membrane. Матеріали Міжнародної науково-практичної конференції, присвяченої 91-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича, (м. Суми, 25–26 травня 2020 р.), 44–45.

He Songtao, Zhou Junguo, Skliar V. H. & Xinxiang (2021). Mechanism of plant adaptation to osmotic stress. «Гончарівські читання»: Матеріали Міжнародної науково-практичної конференції, присвяченої 92-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (м. Суми, 25 травня 2021 р.), 108–109.

He Songtao (2022). Results of the analysis current status of salinization worldwide. Матеріали Всеукраїнської наукової конференції студентів і аспірантів, присвяченої Міжнародному дню студента (м. Суми, 14–18 листопада 2022 р.), 32.

Хе Сунтао (2023). Вплив сольового стресу на розмір та віталітет рослин гарбуза. Матеріали науково-практичної конференції викладачів, аспірантів та студентів Сумського НАУ (м. Суми, 20–25 квітня 2023 р.), 57.

LIST OF CONVENTIONAL ABBREVIATIONS

AMF – arbuscular mycorrhizal fungi

IAI – insoluble acid invertase

SuSy – sucrose synthase

SPS – sucrose phosphate synthase

WW – well-watered

RWC – relative water content

DAP – days after pollination

WW – the well-watered

DI – the deficit irrigation

WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi

DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi

2X – the diploid watermelon

2X/P – the diploid watermelon grafted onto the pumpkin

3X – the triploid watermelon

3X/P – the triploid watermelon grafted onto the pumpkin

MDA – malondialdehyde

P_n – photosynthetic rate

G_s – stomatal conductance

C_i – intercellular CO_2 concentration

T_r – transpiration rate

WUE – water utilization ratio

L_s – stomatal limit

GB – glycine betaine

AFO – active forms of oxygen

LEA – Late Embryogenesis Abundant proteins

Chl – chlorophyll

PS – photo system

ΦPSII – photo system II

PFD – Optical Quantum Flux Densit

Fv/Fm – maximum photochemical efficiency

ETR – electron transport rate

CONTENT

INTRODUCTION

The rationale for choosing the research topic. At the current stage, about 20% of the world's arable lands are subject to salinization [1]. According to FAO, the total area of land affected by salinization exceeds 12 million hectares [2]. Against the background of global climate changes, their area is constantly expanded [3–5]. Soil salinization leads to the loss of up to 50% yield [6]. As a result of the decrease in yield and quality of products [7], which are manifested against the background of soil salinization, the annual losses of the world agriculture are about 12 billion dollars, and this value is still increasing [8]. As a result, salinization becomes one of the powerful factors limiting the sustainable development and modernization of world agriculture [9–12]. These facts and the fact that against the background of the increase in the world population and the shortage of arable land in, the territories, that have undergone salinization become an important reserve land resource [13, 14], studies aimed at elucidating the mechanisms of response and adaptation of plant organisms to salt stress are becoming more and more important [15–17]. These studies should first of all cover the leading agricultural crops, include a number of representatives of the family *Cucurbitaceae*, which are widely cultivated in the world due to its economic, medicinal, edible and ecological values [18–23]. *Cucurbitaceae* are not halophytes, most of them are sensitive to salt stress and usually show obvious inhibition of growth in conditions where the NaCl content in the soil solution reaches 0.1% [24].

Taking into account the above, the issue of clarifying the ecological-physiological, genetic aspects of the response to salt stress of representatives of the *Cucurbitaceae* family and the actual genus *Cucurbita*, the formation of their salt resistance, becomes relevant and managing this trait on the basis of the use of both classical and modern scientific and technical means. Such studies have significant theoretical and practical significance, because they allow to deepen knowledge about the essence of the phenomena of life, about the role of different levels of the organization in ensuring response of plants to the influence of internal and, external factors, to determine the possibility and approaches of managing adaptation processes and, on this basis, for territories, that have undergone salinization, to develop conceptual principles for the

formation of sustainable and productive agrocenoses with the participation of the *Cucurbitaceae* family, including its typical representative: genus *Cucurbita* (pumpkins).

Connection of the research with scientific programmes, plans, and topics. The work was done according to the plans of the research institute work of the Department of Ecology and Botany at Sumy National Agrarian University and according to the plans of the research institute work of Henan University of Science and Technology. In particular, research was supported by grants from the Key Research and Promotion Projects of Henan Province (No.212102110410 & No. 202102110202).

The purpose and objectives of the study. The aim of the research was: to establish mechanisms of response to salt stress and adaptation to it, which are implemented by representatives of the genus *Cucurbita* at different levels of organization, as well as eco-physiological aspects of the formation of salt resistance, quantitative and qualitative characteristics of plants when using grafting technology.

To achieve of the set goal were formulated the following tasks:

1. To evaluate the influence of salt stress on the morphostructure and habit of pumpkin individuals, as well as their vitality and representation among the studied plants of individuals of different vitality classes.
2. To evaluate the effect of different concentrations of salts on the degree of salt damage to pumpkin.
3. To find out the influence of salt stress on indicators related to the flow of photosynthesis and water exchange of pumpkin
4. To study the processes related to the effect of salt stress on cells and their membranes
5. To find out the peculiarities and regularities of the metabolism of leading organic substances in the body of salt stress and adaptation to it.
6. To establish the nature of changes in the ionic composition of plants, which is manifested in the response and adaptation system to salt stress.
7. To study the manifestation of individual varietal characteristics of pumpkin plants in response to salt stress

8. To identify the WRKY gene family and analyse its expression in response to salt stress in pumpkin.
9. To study the possibility of applying the latest methods for rapid functional analysis of genes, including those determining salt tolerance.
10. To find out the essence of eco-physiological interactions and transformations that are manifested in plants when using salt-resistant pumpkin rootstocks in the cultivation of melon crops and their adaptation to salt stress.
11. To study the influence of symbiotic relationships of pumpkins with arbuscular mycorrhizal fungi on eco-physiological characteristics of grafted plants.

The object of the research. Adaptation processes and ecological-physiological interactions manifested in plants of the family *Cucurbitaceae*

The subject of the research. Adaptations of pumpkin to salt stress at different levels of the organization and the use of salt-tolerant pumpkin rootstock for cultivation of melon cultures.

Research methods. The work was performed on the basis of the application of theoretical (analysis, synthesis, explanation, generalization, a mathematical and statistical) and empirical (experiment, measurement, comparison) research methods. Experimental field and laboratory ecological-physiological and laboratory genetic studies were carried out, chemical, spectrometric, fluorescent, morphometric, and vitality analyses were applied, and among mathematical and statistical calculations, point estimation of statistical series and dispersion analysis were used.

The scientific novelty of the obtained results. For the first time, based on the results of observations during the entire growing season, the influence of salt stress on a complex of quantitative and qualitative morphological characteristics of pumpkin plants was studied. The features and regularities of the accumulation of ions of mineral substances in the vegetative and generative organs of pumpkins under conditions of salt stress have been established. Physiological and biochemical transformations that appear in pumpkins against the background of salt stress were comprehensively investigated and clarified.

There have been screened 12 WRKY family transcription factors in pumpkin in response to salt stress, and analysed their phylogenetic relationships, spatio-temporal

expression patterns, tissue-specific expression characteristics, and transcriptional activities under salt stress in detail.

For the first time, the eco-physiological interactions and transformations that occur during the cultivation of *Cucurbitaceae* using grafting technology and the use of arbuscular mycorrhizal fungi were investigated and clarified.

The methodological aspects of the assessment of salt stress impact assessment during ontogenesis of pumpkin have been improved and the technique of vitality analysis was used to assess the condition of pumpkin plants.

The efficient transient transformation system for the study of gene function and protein subcellular localization in pumpkin have been developed.

The further development of the theoretical and practical principles of the use of salt-tolerant pumpkin rootstock for cultivation of watermelon have been gained.

The practical significance of the obtained results. The materials of the dissertation work are used in the educational process of the Department of Ecology and Botany of Sumy National Agrarian University when teaching such disciplines as “Ecological Physiology of plants”, “Agroecology”, “Biology”.

The doctoral candidate’s contribution. The dissertation student processed the sources of literature independently, mastered the relevant research methods and conducted the necessary experiments. The part of the experiments were carried out together with employee’s research institutions of the Republic of China: Henan Institute of Science and Technology, Henan Province Engineering Research Center of Horticultural Plant Resource Utilization and Germplasm Enhancement, College of Horticulture and Landscape Architecture. The share of personal participation of the acquirer in joint publications is more than 60%. The interpretation and the generalization of the results and the preparation of the publications were carried out with the participation of the scientific supervisor.

Approbation of the dissertation results. The main results position theses were considered and discussed at 6 conferences of different rank. In particular, they are presented on the following international scientific and scientific and practical conferences: «Goncharivskyi reading» (Sumy, 2019, 2020, 2021). Also, the results of

dissertation studies are presented on at scientific and practical conferences of Sumy National Agrarian University (Sumy, 2019, 2022, 2023).

Publications. According to the materials theses 12 scientific papers were published: three articles were published in a scientific journal, included in the database of Scopus, Web of Science, three articles were published in professionals scientific journals of Ukraine, six publications were published in materials and abstracts of reports at international and national conferences.

Structure and scope of the dissertation. The materials of the dissertation are presented on 182 pages. The dissertation consists of an annotation, a list of symbols, introduction, three chapters of the main part, conclusions, the list of sources and five appendices. The list of references includes 352 sources.

CHAPTER 1
RESPONSE AND ADAPTATION OF *CUCURBITACEAE* TO SALT STRESS, WAYS OF INCREASING THEIR SALT RESISTANCE (LITERATURE REVIEW)

1.1. Eco-physiological and biochemical aspects of the response of plants to soil salinin and salt stress

Soil salinization is the process of easily soluble salts accumulation in the soil or the surface layer of the soil. Depending on their composition, several main types of salinization are distinguished: chloride, sulfate, sodium [7, 24, 25]. It is the result of natural and (or) anthropogenic factors [24, 26–29]. At the same time, the arid climate impact the spread of salinization among the former, and among the latter non-compliance with technologies and environmental standards during management, for example, during irrigation [30, 31]. Establishing the mechanisms underlying the adaptation of plants to salinization is an urgent scientific problem, important from both a theoretical and a practical point of view. Currently, many scientists are working on it and, accordingly, a significant amount of data has already been accumulated.

In the sources, it is noted that osmotic stress can occur in plants based on salinization, and at the level of an individual in general – ion imbalance (Fig. 1.1, 1.2) [32–36]. The plant damages by salt ions are divided into direct and indirect. The first of them is usually caused by osmotic stress and ion toxicity, and the second is manifested in secondary reactions that occur under the effects of direct damage [37, 38].

Osmotic stress effects cell growth and metabolic transformations. It can rapidly change water-related parameters, including relative water content, water potential, and osmotic potential in leaves. There is a decrease in the rate of leaf growth, a change in the enzyme activity and pigment content, the state of the stomata and the process of photosynthesis, and the growth of shoots is inhibited [33, 39–42].

The accumulation of large amounts of salt ions causes plants to use more energy to absorb water from the soil and maintain internal homeostasis [43]. The accumulation of toxic ions under the influence of salt stress is the main cause of plant growth inhibition [44].

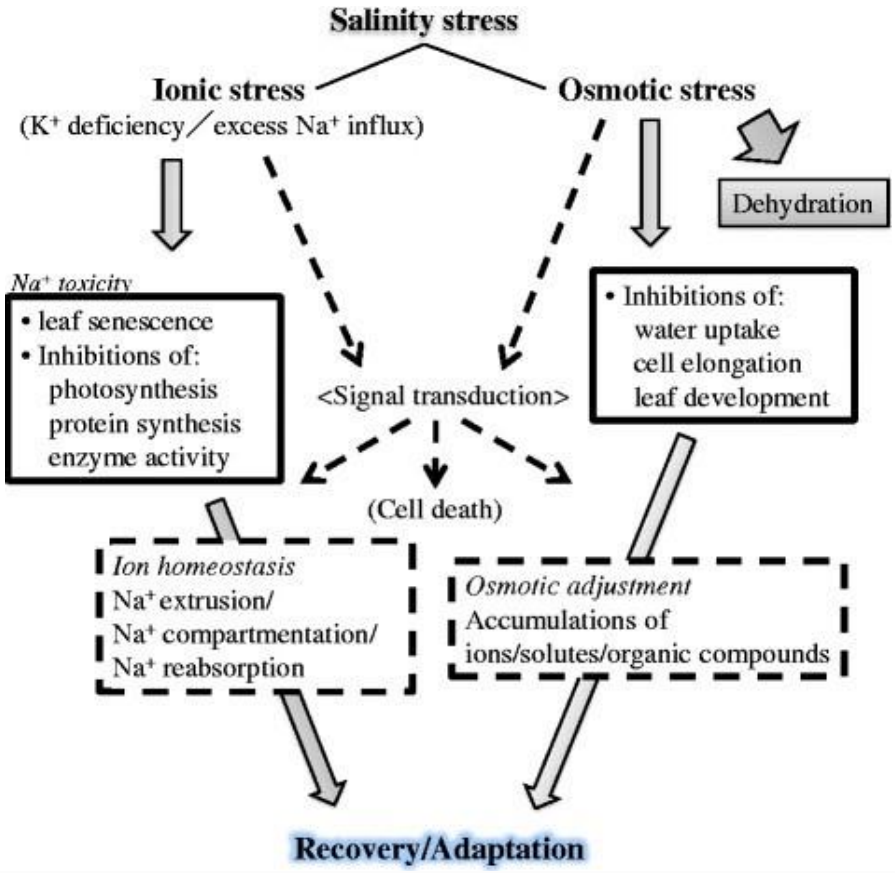


Fig.1.1. A schematic summary of the stresses that plants suffer under high salinity growth condition and the corresponding responses that plants use in order to survive these detrimental effects [35]

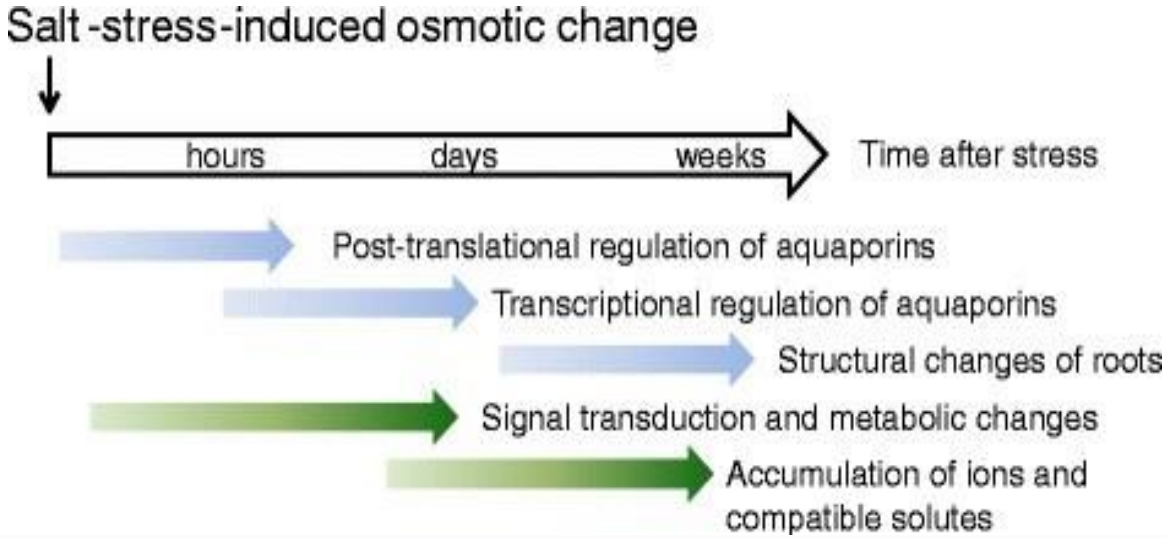


Fig.1.2. Timeline of regulations and changes in simplistic water-related functions of a plant cell after osmotic changes. Blue arrows indicate functions included in the regulation of the water permeability (conductance) of roots, and green arrows indicate functions of the cellular osmotic adjustment [35]

Salt stress is most often caused by NaCl. Compared to the accumulation of Na^+ in the roots, its concentration in the leaves of the plant causes more damage to it, because it is accompanied by their etiolation and necrosis [37, 45–47].

Under the salt stress impact, the accumulation of reactive oxygen species (ROS) is activated and oxidative damage to cells and their structures occurs [48]. These oxygen species negatively affect protein stability, especially the status of amino acids such as tyrosine, phenylalanine, tryptophan, and cysteine [49] and can inhibit enzyme activity [50]. Membrane lipid peroxidation is considered as a marker of oxidative damage in plants under salt stress conditions [51]. That is, the mechanisms of the salt stress effect on plants, as well as their response to such an effect, are quite powerful and diverse [52].

Depending on the ability to grow on saline soils, plants are traditionally divided into halophytes and glycophytes [8, 53]. Due to the development of molecular biology, studies of the plant adaptation mechanism to stress moved from the physiological and ecological level to the molecular level. Modern research is aimed not only at explaining the mechanism of plant adaptation to stress, but also at obtaining various gene resistance to crop breeding [54].

Plant cytoplasm cannot resist to high salt concentrations, so they need to limit excess salt entering the vacuole, other compartments, and tissues. So far, five salt resistance genes have been identified: SOS5, SOS4, SOS3, SOS2, and SOS1 [55, 56]. The SOS1 gene encodes a 127 kDa polypeptide containing 1146 amino acid residues [54, 56]. SOS1 protects plasma membrane K^+ ion transport during salt stress [56]. The SOS2 gene encodes a serine/threonine protein kinase containing 446 amino acids [55], with a molecular weight of about 51 kDa. SOS2 interacting with Na^+ and H^+ ions effects their exchange activity and directs excess Na^+ to vacuolar regions, thus contributing to ion balance [57]. In addition, [58] found that SOS2 detects environmental changes by differentially regulating SOS1 and H^+ and Ca^{2+} ion exchange. The SOS3 gene encodes a calcium-binding protein [59]. When the level of Ca^{2+} changes, SOS3 interacts with SOS2. Through SOS3, the SOS3/SOS2 protein kinase complex activates SOS1, and activated SOS1 excretes an excess of Na^+, thereby contributing to the maintenance of Na^+ balance [59, 60]. Thus, three genes,

SOSI, SOS2, and SOS3, are actively involved in signal transduction related to the maintenance of intracellular ion balance [59, 60–63].

The SOS4 gene is widely expressed in all plant tissues and is an important cofactor for many intracellular enzymes, and it regulates the activity of specific ion transporters in cells [64]. Compared with natural flora plants, genetically modified plants containing SOS4 accumulate more Na^+ and store less K^+ [65]. Thus, SOS4 becomes a new determining factor that powerfully regulates the balance of Na^+ and K^+, ensuring plant resistance to salinization. The SOS5 gene encodes a polypeptide containing 420 amino acid residues. It is concentrated on the outer surface of the plasma membrane [66]. The SOS5 gene may play a certain role in intercellular adhesion, maintenance of cell wall integrity, and cell resistance under salt stress [67, 68].

Oxidative stress also occurs based on the joint action of salinization and drought. During salinization stress, as a result of active stomatal closure, the availability of atmospheric CO_2 is reduced, as well as the consumption of NADP and a chain reaction with the formation of more harmful oxygen free radicals is initiated [69]. Metabolic disturbance is observed due to oxidative damage to lipids, proteins, and nucleic acids (McCord, 2000). Opposite these negative effects, an antioxidant system of enzyme protection is formed in plants, as well as a non-enzymatic system of molecular protection, the aggregates of which are glutathione, ascorbic acid, carotenoids, etc. [70].

To overcome oxidative stress, plants remove excess active forms of oxygen (AFO) by activating antioxidant enzymes. The degree of oxidative damage is directly determined by the properties of the plant antioxidant system [71, 72]. Research results prove the existence of a correlation between antioxidant capacity and salt stress resistance in citrus fruit [73], wheat [74], beans [75], rice [76], purslane [77]. Transgenic plants overexpressing AFO scavenging enzymes have increased resistance to osmotic, temperature, and oxidative stress [78, 79].

Under normal conditions, AFO production and its neutralization in plants are in dynamic equilibrium. During the salt stress, this balance is broken. Accordingly, the

AFO absorbtion system plays a very important role in the physiology of plant salt resistance [80, 81].

The main result of salt stress is the loss of intracellular water. Plants accumulate many metabolites, and these "adaptive (osmotic) solutions" in the cytoplasm increase hyperosmotic resistance to water loss caused by salt stress. The high osmotic concentration balances the high concentration of extracellular salt, on the one hand, and neutralizes the high concentration of Na^+ and Cl^- ions in the vacuole, on the other hand [8].

Such substances as proline, betaine, polyamine, glycerol, sorbitol and inosyt, as well as some soluble sugars and soluble proteins are involved in ensuring organic osmoregulation. Wherein, proline plays a leading role [82-84]. There are two main mechanisms of proline accumulation under the influence of salt stress: firstly, it is the activation of biosynthesis [85]; secondly, reducing its oxidative degradation [86]. Increased proline synthesis under conditions of drought or salt stress can mitigate cytoplasmic acidosis and maintain the $NADP^+$/NADPH ratio at the required metabolic level [87]. It was proved that salt stress significantly increased the accumulation of proline in the leaves of two rice varieties, and it contributed to their osmotic adaptation and increased resistance to salinization [88].

In plants and many other organisms, including bacteria, algae and fungi, animals synthesize and accumulate glycine betaine (GB) in response to some abiotic stresses. It also protects higher plants from salt/osmotic stress by providing osmotic function. The correlation between the protective effect of GB and the antioxidant defence system is registered in rice [88], in tomatoes [89], in wheat [90, 91], in tobacco [92].

Plants have the ability to quickly perceive and actively adapt to changes of the external environment. This strategy of perception and active adaptation to environmental changes is the result of stress signalling, which includes three stages: perception of stress signals by the plant, transmission of stress signals, their recognition and response to them [93]. Nowadays, the signal transmission ways, related to plant salt resistance are known, which mainly include SOS signalling way, protein kinase signalling way, protein phosphatase signalling way, Ca^{2+} and calmodulin signalling way, osmotic stress signalling way, etc. Ca^{2+} is an active

messenger molecule in the cells of higher plants. Cold, drought, and salt stress can cause changes in the cytosolic Ca^{2+} concentration, which is a signal for further unfolding events [94].

Membrane Na^+/H^+ antitransporters located in the vacuolar membrane and plasma membrane, respectively, play an important role in the formation of plant resistance to salt stress [95–98].

Under the influence of salt stress in plants, some pathways of protein synthesis are inhibited, but protective proteins are synthesized. The synthesis of salt stress proteins in cells is important [99–103]. In vegetative tissues dehydrated by stresses such as drought, low temperature, and salinity, proteins called LEA (Late Embryogenesis Abundant) proteins are synthesized [104]. The LEA protein may function as a dehydration defence agent to protect cells from reduced water potential during seed maturation, desiccation, and osmotic stress. The stress resistance of transgenic plants is positively correlated with the accumulation of the LEA protein, which became direct evidence of the main role of the LEA protein in the process of plant salt tolerance [105].

Plant salt tolerance is a complex trait controlled by several genes. The role of one gene in the process of ensuring plant resistance to salt is limited. Currently, scientific research has entered the post-genomic era, that is, the era of the functional genome. In the post-genomic era, proteomics is gradually becoming an important method for studying gene function [106]. Since proteins are involved in most life processes, proteomic studies help to understand in more detail the biological functions of cells at the molecular level, including the prostheses associated with ensuring salt tolerance [107].

1.2. *Cucurbitaceae* family: leading traits and data on responses to salt stress

In the modern world, the issue of providing the population with food products is one of the most urgent. According to some data, approximately 40 million people die annually on Earth from hunger and its consequences. This problem, which is also becoming more acute against the background of the decrease in the area of land

suitable for cultivation, forces us to look for ways to increase the yield of plants and the productivity of various branches of agriculture [108–114].

A component of the problem of providing the population with food products is the satisfaction of people's needs in vegetables, at the expense of products obtained in the process of growing *Cucurbitaceae* representatives as well. Data on the number of genera and species belonging to this family differ significantly: the number of genera is indicated in the range of 90–130, and the number of species is 600–1100. *Cucurbitaceae* is mainly represented by perennial and annual herbs common in warm climates. A common botanical feature of the family is a liana-like life form. The fruits of many representatives of the family (melons, cucumbers, pumpkins, watermelons, zucchini, squash, and a number of others) are edible and very popular with the population as a food product [18, 19, 23].

Considering the above, it is quite natural that now considerable attention is paid to improving technologies, applying innovations in the cultivation of plants of the *Cucurbitaceae* family [115-120] and carrying out selection work for them [121-128]. Recently, attention to the introduction of rare plants of this family has been growing [129, 130].

Pumpkin is a melon crop of the genus *Cucurbita* L, which includes 21 species, five of which are cultivated (*C. pepo* L., *C. maxima* Duch., *C. moschata* Duch., *C. mixta* Pang., *C. ficifolia* Bouche) [131–136]. The wide and diverse use of pumpkins is determined not only by their high productivity [137], but also by biological [138, 139], food [132–136] and medicinal properties [23, 137–139]. Modern medical research has found that pumpkin can prevent diabetes, hypertension, coronary heart disease, cerebrovascular disease, etc. Pumpkin contains a variety of nutrients, such as carbohydrate, protein, cysteine, ascorbic acid, etc. Pumpkin fruit is rich in a variety of vitamins, amino acids and minerals. Pumpkin seeds contain a variety of healthy active ingredients, such as antioxidants and antitumor chemicals [20, 21]. Pumpkin has also been shown to have a unique ability to take up harmful pollutants from soil [22]. Members of the genus *Cucurbita* are believed to originate from the American continent [133, 140, 141].

Plants of the Cucurbitaceae family are not halophytes, however, as a result of their popularity and cultivation in various regions, including those that are subject to salinization or have a potential danger of the spread of saline soils, the study of the characteristics and regularities of the response of plants of this family to salinity is an urgent scientific problem.

Scientists paid considerable attention to the effect of salt stress on the germination of melon seeds. The obtained results indicate that the treatment of their seeds with low concentrations of salts (primarily NaCl) can even promote the activation of germination, while high concentrations usually have an inhibitory effect [142–146].

Studies on its salt tolerance have been reported from the germination stage to the seedling stage in terms of growth and osmoregulation [147, 148].

In the degree of sensitivity to the negative impact of increased salt concentrations on the state of plants of the *Cucurbitaceae* family, there is a manifestation of both species and varietal characteristics [149–152].

According to the results of studies conducted on melon, it was established that its seeds, pre-treated with salt, really germinated earlier, and the seedlings had a higher dry weight and content of total sugar and proline. That is, such seedlings had a higher salt tolerance [153]. In addition, plants treated with salt had higher chlorophyll, K^+ and Ca^{2+} [154]. At the same time, in other experiments, a decrease in leaf area, plant weight, and accumulation of dry matter were recorded in melons under the influence of salt stress [155].

In cucumbers, the content of chlorophyll and carotenoids in seedling leaves increased under short-term salt stress [156]. Salt stress was accompanied by an increase in the rate of synthesis of O_2^-, an increase in the activity of peroxidase, the content of H_2O_2, malondialdehyde and, as a result, – a slowdown in plant growth [143, 157, 158].

When studying zucchini treated with different concentrations of salt, it was found that as the concentration of salt solution increases in the leaves of these plants, the content of Fe, Mn, and Zn increases, and Cu decreases. At the same time, the content of Fe and soluble Mn in the fruits also increases [159].

1.3. Genetic research of salt resistance plants

Plant salt tolerance is a complex trait controlled by genes. Accordingly, the study of processes that are regulated at the genetic level is an important component of research aimed at establishing the mechanisms of ensuring plant salt tolerance and its regulation [72, 106]. Genetic engineering plays an important role in this system of measures. Genetic engineering can improve crops by exploiting important genes and engineering transgenic plants with targeting properties [160].

Transcription factor is a kind of protein molecule with special structure and function of regulating gene expression. There are two kinds of transcription factors in plants. One is a non-specific transcription factor, which can non-selectively regulate the transcriptional expression of genes, such as HvCBF2 (C-repeat/DRE binding factor 2) in barley [161]. The other is specific transcription factors, which can selectively regulate the transcriptional expression of certain or certain genes, such as WRKY, bHLH, bZIP, MYB, NAC, HMG, HSF, zinc-finger protein, AP2/ERF (ethylene response factor), etc. They play an important and unique role in regulating plant specificity [162, 163]. Among them, WRKY gene family is one of the largest transcription factor families in higher plants, which is found in the whole plant lineage. Ishiguro et al. [164] cloned the world's first WRKY gene SPF1 (Swet potato factor 1) from sweet potato, and subsequently successfully isolated and identified WRKY transcription factors in a variety of plants. The WRKY gene family has shown a process of gene expansion during its evolution from one or a few genes in green algae, to more than 30 genes in the earliest terrestrial mosses, to more than 100 genes in higher plants.

Rushton et al. [165] identified WRKY1, WRKY2, and WRKY3 from parsley, named WRKY (pronounced "worky"), and demonstrated for the first time that WRKY proteins play an important role in regulating plant responses to pathogens. It also plays a potential role in regulating the expression of sucrose (SPF1) or germination (ABF1 and ABF2) genes. Since the publication of WRKY transcription factors in 2000, scientists have made significant progress in the study of WRKY proteins in the past decade.

WRKY transcription factors have very significant structural characteristics. The protein structure of WRKY transcription factors basically contains 1-2 WRKY domains, which are DNA binding domains (DBD), composed of about 60 highly conserved amino acid residues, including heptapeptide located at the N-terminal and zinc finger structure located at the C-terminal. The N-terminal heptapeptide WRKYGQK is absolutely conserved and is the core sequence, while the C-terminal sequence is composed of C2H2 (C-X4-5-C-X22-23-H-X-H) or C2HC (C-X7-C-X23-H-X-C) type zinc finger structure [166, 167, 168]. WRKY transcription factors can specifically bind to the cis-acting element TTGAC (C/T) nucleotide sequence (W-box) in the promoter region of target genes through WRKY domain to activate or inhibit transcription and then regulate the expression of downstream genes [169, 170]. Yamasaki et al. [171] reported that the WRKY4 domain of Arabidopsis thaliana consists of a four-stranded β-lamellar, in which a zinc-binding pocket is formed by conserved cysteine/histidine (Cys/His) residues at the C-terminus of β-lamellar, and WRKYGQK residues correspond to the N-terminal chain of the sequence. It is twisted by the Gly residue in the middle of the sequence, allowing extensive hydrophobic interactions involving Trp (tryptophan) residues, thereby contributing to the structural stability of the β-chain. The WRKYGQK motif of the β chain can contact a region of approximately 6 bp, which is largely consistent with the length of the W-box (TTGACY). These results indicate that the WRKYGQK motif can bind specifically to the W-box structure of the promoter region of the target gene.

The domain of WRKY transcription factor has many variations in different plants. Rice WRKY family members have 19 variants of WRKY domains, among which WRKYGEK and WRKYGKK are common mutants shared by 7 domains and 5 domains [172]. Among the 53 CiWRKY genes identified from the transcriptome data of Caragana intercala, the CiWRKY protein contains both the highly conserved WRKYGQK motif and two variant WRKYGKK and WKKYEEK motifs [173]. Some studies have shown that WRKYGQK sequence mutation can significantly reduce the binding activity of WRKY transcription factors to DNA. In the domain of tobacco NtWRKY transcription factor, the C-terminal domain has strong activity, while the N-terminal WRKY domain has weak binding activity with W-box. The conserved

cysteine and histidine residues in the C2H2 zinc finger motif are replaced. It can also destroy WRKY transcription factors binding to DNA [168].

In addition to the WRKY domain, WRKY transcription factors contain other domains, Including TIR-NBS-LRR (Toll/interleukin-1 receptor-nucleotide binding site-leucine-rich repeat), kinase domain, proline rich region, glutamine rich region, serine - threonine-rich area, leucine zipper, nuclear localization domain, etc. [174]. Arabidopsis AtWRKY7 contains both a WRKY domain and a calmodulin CAM-binding domain [175]. The diverse domains of WRKY transcription factors indicate that WRKY transcription factors with special structures can play important, special functional roles in the regulation of gene expression.

Before obtaining the complete WRKY gene family sequence from plants, Eulgem et al. [166] divided WRKY family members into three subfamilies: GroupI, GroupII and GroupIII. Group I contains two WRKY conserved domains, and its C-terminal zinc finger structure is C2H2 type. Group II contains only one WRKY conserved domain, and the zinc finger structure is the same as that of class I, which is C2H2 type. Group III zinc finger structure is C2HC-type and contains a WRKY conserved domain. However, the structure types of a few WRKY proteins do not match these three types of features, such as AtWRKY10, whose structure may be that the N-terminal WRKY conserved domain has been lost and only contains one WRKY conserved domain. The protein structure characteristics were similar to but inconsistent with those of Group I [167]. For example, the C terminus of WRKY protein in apple is classified as Group IV because it lacks a complete zinc finger-like protein structure [176]. Zhang et al. [172] believed that Eulgem et al. 's classification of Arabidopsis WRKY family was not entirely based on phylogenetic analysis. In order to reflect the evolution process of WRKY domain, the WRKY transcription factors were divided into I, IIa+Iib, Iic, IId+IIe and III by phylogenetic analysis. And category II is not a separate category. This was subsequently confirmed by Tamura et al. [177] through pure phylogenetic data analysis. The evolutionary analysis of WRKY transcription factors is important for understanding the overall mechanism of plant biodiversity, as well as the specific functions played by WRKY genes in plant regulatory networks.

Many transcription factors (WRKY, AP2/EREBP, bHLH, C2H2 and ARF) have roles in regulating plant development and dwarfing. Of these, the most known are the WRKY family [178, 179]. WRKY transcription factors can induce plant dwarfing [180]. Overexpression of OsWRKY11 reduced the height of transgenic rice plants, resulting in short stature [181, 182]. The dwarfing phenotype is mainly caused by changes in fine cell division and cell elongation, which are regulated by cytokinin (CTK), auxin (IAA), gibberellin (GA) and brassinosteroid (BR) [183]. IAA, CTK and GA are widely believed to play an important role in plant dwarfing [184-186]. In addition, BR also plays an important role in plant dwarfing. In Arabidopsis thaliana, cpd, cbb3, dwf4 mutants and BR6ox1, BR6ox2 double mutants with BR biosynthesis gene defects all show dwarfing phenotype [183] Zheng et al. [187] showed that WRKY could directly regulate BR biosynthesis. Apple transcription factor MdWRKY9 restricts the transcription of MdDWF4 synthetase by directly inhibiting brassinosteroid, reduces the production of BR, and positively regulates plant short stature. These results demonstrate that WRKY transcription factors play an important role in the regulation of plant development and dwarfing.

WRKY genes have been reported to play a role in the regulation of plant hormone synthesis [181, 182]. Some WRKY genes affect root structure by affecting plant hormone signaling or gene expression. Overexpression of OsWRKY31 results in fewer and shorter lateral roots than wild-type, possibly by interfering with auxin response or transport [183].

Similar studies have shown that OsWRKY28 loss-of-function mutants may affect root growth by reducing the expression of JA biosynthetic genes [184]. In addition, ethylene (ETH) can induce the formation of root hairs and adventitious roots during root development. In the wheat experiment, the 1-amino-cyclopropen-1-carboxylate synthase gene synthesized by ethylene was down-regulated in the overexpression line TawrKY51-OE, but up-regulated in the gene silencing Tawrky51-Rnai line. Further studies revealed that TaWRKY51 was a negative regulator of ETH synthesis. TaWRKY51 inhibits the expression of ETH synthesis gene ACS by binding to W-box cis-elements present in the promoter region, which coordinates ethylene synthesis and lateral root formation in wheat [185]. These results suggest that WRKY transcription

factors can regulate the synthesis of corresponding plant hormones, and then regulate root development through these plant hormones.

WRKY transcription factors play an important regulatory role in the physiological pathway of fruit ripening. Analysis of expression patterns showed that nearly 60% of CaWRKY was expressed in the ripening process of pepper [186]. ClWRKY was highly expressed in watermelon fruit tissues [187]. WRKY transcription factors related to ripening process were also successfully screened in avocado [188] and strawberry, indicating that WRKY transcription factors may play a regulatory role in fruit ripening process. Among them, FaWRKY transcription factor is involved in abscisic acid (ABA) signaling pathway and promotes strawberry fruit ripening by regulating ABA synthesis [189].

WRKY transcription factors are also involved in the regulation of leaf senescence. WRKY transcription factors are the second-largest family of transcription factors in the ageing transcriptome of Arabidopsis [190]. AtWRKY6 was significantly upregulated during ageing. By analysing the target genes of AtWRKY6, it was confirmed that ageing induced the expression of receptor kinase and receptor-like kinase (SIRK/FRK1) genes. SIRK/FRK1 encodes a receptor-like protein kinase that is strongly and specifically induced during leaf senescence [191, 192]. In other species, WRKY genes (including GhWRKY42, CiWRKY40-4, and BrWRKY6) that regulate ageing have been identified. For example, overexpression of GhWRKY42 leads to increased expression of senescence related genes and promotes premature leaf senescence [193]. CiWRKY40-4 overexpression in Arabidopsis inhibits leaf senescence and is a negative regulator of senescence. GA can inhibit leaf senescence, while BrWRKY6 binds to the promoters of ageing-related genes BrSAG12, BrNYCl and BrSGR1 through W-box cis-elements, and inhibits the expression of GA biosynthetic genes BrKAO2 and BrGA20ox2 accelerated leaf senescence [194].

The expression of WRKY transcription factors is strongly and rapidly induced by a variety of environmental and internal factors, especially those related to biological stress. Plant responses to biological stress depend on a wide variety of receptor proteins present in the cell membrane and inside the cell. In general, receptors on cell membranes initiate resistance responses by recognizing characteristic sequences

conserved on pathogens. Such conserved feature sequences are referred to as Pathogen associated molecular patterns (PAMPs) for short. The receptors that recognize these sequences are called Pattern recognition receptor (PRR). The resistance responses mediated by them are known as PAMP triggered immune responses (PTI) [195, 196]. In addition, receptor proteins inside cells are mostly encoded by NBS (Nucleotide binding site) –LRR (Leucine-rich repeat) disease-resistant genes, which directly or indirectly recognize effectors released into cells by pathogens and initiate disease-resistant responses. This response is known as Effector triggered immunity (ETI). Both reactions are mediated by WRKY transcription factors [195–197].

WRKY proteins can activate or repress transcription and are generally rich in potential transcriptional activating and repressing domains. Some WRKY factors have two functions. For example, in yeast, AtWRKY53 activates or represses reporter gene transcription depending on the sequence upstream and downstream of the promoter. AtWRKY6 negatively autoregulates its own promoter while transcriptionally activating the SIRK gene, which encodes a receptor-like protein kinase associated with ageing. Transient expression studies showed that OsWRKY72 and OsWRKY77 were both ABA signalling activators and GA signalling inhibitors in aleurone cells [198].

Studies have shown that many genes are activated by WRKY factors associated with their promoters. BhWRKY1 binds to the W-box of the BhGolS1 (galactol synthetase) promoter and activates transcriptional BhGolS1 expression, which can improve drought tolerance in Arabidopsis [199]. AtWRKY50 can interact with TGA2 or TGA5 and bind to the W-box of PR1 promoter, synergistically activating PR1 expression and enhancing Arabidopsis resistance [200].

It is involved in the regulation of WRKY transcription factor binding activity. For example, in Arabidopsis, MEKK1 protein kinase is a bifunctional protein that can both bind to the WRKY53 promoter at a site upstream of the W-box (WP1) and induce phosphorylation of the AtWRKY53 transcription factor in the Arabidopsis ageing-induced signalling pathway. It promotes the binding of AtWRKY53 transcription factor and its own encoding gene promoter to regulate ageing in Arabidopsis [201]. WRKY46 transcription factor binds to the promoter of PAMP responsive gene NHL10,

increases the expression of NHL10 gene, and regulates the defence response against bacterial flagellin pathogens in Arabidopsis [202].

Another mechanism of WRKY function is through small RNA (smRNA) (microrNA, miRNA and small dry RNA, siRNA), which have become the basic mode of regulating gene expression. Since the predicted targets of multiple mirnas encode WRKY transcription factors, WRKY can not only regulate the amount of smRNA, but also the WRKY transcription factors themselves are smRNA targeting factors [203]. One of the modes of action is that the target gene is bound to the regulatory site of the promoter by other transcription factors, which prevents the binding of the own transcription factor to the target gene promoter. For example, PcWRKY1 can bind to the W-box of the PcPR10 gene promoter, mitogen-activated protein kinase can modify the bound PcWRKY1 transcription factor in the nucleus, and this modification leads to allosteric release of PcWRKY1 transcription factor [204].

Another form of transcriptional repression is to prevent transcription by altering the higher structures of DNA or histones. For example, epigenetically modified DNA methylation and demethylation, histone acetylation and deacetylation are key mechanisms that induce stress transcription to turn off or on. DNA methylation can cause changes in the way DNA interacts with proteins, thereby inhibiting gene expression. For example, DNA methylation at the promoters of WRKY50 and WRKY72 in rice leaf tissues decreased the expression levels of WRKY50 and WRKY72 [205]. On the contrary, DNA demethylation can realize the deinhibition process of WRKY transcription factors, so that the inhibitory effect of WRKY transcription factors can be converted to the activation effect. The promoter region of DBR2 was demethylated at four CG–, four CHH– and two CHG– sites, leading to upregulation of DBR2, a key regulatory gene for artemisinin biosynthesis, indicating that demethylation at the WRKY promoter promoted the expression of ABI5 artemisinin [206]. Similarly, deacetylation of histones inhibits transcription factors from binding specifically to DNA-binding sites, whereas acetylation of histones has the opposite effect. Histone deacetylase 19 (HDA19) inhibits the transcription of AtWRKY38 and AtWRKY62 by removing acetyl groups from histone tails, and negatively regulates basal defence [207]. Another acetylation study showed that the

acetylation of histone H3K9 (lysine 9 of histone H3) on the WRKY40 promoter in Arabidopsis increased WRKY40 gene expression and enhanced Arabidopsis resistance to Fusarium [208].

A characteristic feature of the WRKY signalling network is regulation through autoregulation of WRKY transcription factors interacting with their own promoters and cross-regulation of the actions of other WRKY transcription factors, which is achieved by identifying and binding to W-box promoters in target genes. For example, CaWRKY40b shows positive feedback regulation at the transcriptional level by directly targeting the W-box in its own promoter, thus achieving self-regulation of WRKY transcription factors. Parsley PcWRKY1 has a conserved arrangement of three synergistic W-boxes in the promoter [209]. After PAMP induction, PcWRKY1 transcription products increased [210]. Chromatin immunoprecipitation analysis showed that these three W-boxes were bound by WRKY transcription factors. However, when PcWRKY1 binds to its own promoter, the transcription of PcWRKY1 is down-regulated, indicating that the W-box at the promoter site of PcWRKY1 is bound by other WRKY transcription factors to activate transcription [211]. Based on bioinformatics and functional studies of plant promoters, it was found that many WRKY gene promoters were statistically enriched in W-boxes [212]. For example, in parsley PcWRKY1, there are multiple W-boxes, which have a synergistic effect on transcription. Transcription of barley HvWRKY38 requires two adjacent W-boxes to bind effectively [213]. Two rice OsWRKY45-DBD molecules exchange β4-β5 strands to form dimers, which contain two DNA-binding domains that interact with W-boxes [214].

Mitogen-activated protein kinase (MAPK) exists in all eukaryotes and is a highly conserved module. In plants, the MAPK pathway is involved in the regulation of development, growth, programmed cell death, and response to various environmental stimuli [215]. The MAPK signalling level connects multiple phosphorylation processes to upstream receptors and downstream targets, amplifies and transduces pathogen-derived signals sensed by membrane receptors through phosphorylation, and changes the expression of related genes by these signals [216].

MAPK signalling pathway responds to MTI (immunity triggered by MAMP, a conserved molecule that recognizes microorganisms) or PTI defence signalling pathway in plants. MAMP and PAMP can be sensed by intracellular MAPK signalling cascade in plants during immune response, and the induction of WRKY transcription factors is stimulated. In Arabidopsis, the transcription factor AtWrky33 forms MAMP or PAMP complexes with MAP kinase 4 (MPK4) in the absence of pathogen infection [217]. In a subsequent study, phosphoprotein migration and transfer assay showed that AtWrky33 could be phosphorylated by MPK3/MPK6 and promoted AtWrky33 to regulate the biosynthesis of phytoantitoxin [218]. Conversely, the loss of MPK3/MPK6 phosphorylation site in WRKY34 affects WRKY34 function in vivo [219]. OsWRKY53 functions as a negative feedback regulator of MPK3/ MPK6, thereby playing an early inhibitory role in inducing defence [220]. The positive regulatory loop composed of MPK3/MPK6, Wrky33, AGD2-like defence response protein 1 (ALD1) and piperidolic acid (PiP) exists in the induction of systemic acquired resistance (SAR), suggesting that there are different SAR activation pathways at the level of PiP biosynthesis [221].

ETI of plant defence signalling pathway is specific resistance based on the recognition of effector proteins by resistant R proteins. After a pathogen invades plants, plants secrete resistant R proteins to recognize pathogen effector proteins, WRKY transcription factors interact with resistant R proteins to form protein complexes, and relieve the inhibition of basic defence pathways [222]. In barley immunity against powdery mildew, the ETI immune pathway is involved. The resistance R protein MLA in the cytoplasm can recognize the powdery mildew effector AVR10 and bind to HvWRKY1 and HvWRKY2 in the nucleus to relieve the inhibitory effect of HVWRKY1/2 on disease resistance. Thus, the purpose of disease resistance is achieving [223]. Typical chimeric proteins, such as R protein NBS-LRR and WRKY transcription factor chimeric proteins, have been found to play an important role in immune regulation. For example, AtWRKY52/RRS1, a chimeric protein formed by WRKY transcription factors interacting with RRS1 (a NBS-LRR protein), provides immunity to bacterial pathogens through interaction with the bacterial effector PopP2 [224, 225]. These studies demonstrated that WRKY transcription factors play an

important regulatory role in the immune system of ETI by binding to disease-resistant R proteins, recognizing effector proteins produced by pathogenic microorganisms and triggering specific defence responses in plants.

Proteins, especially regulatory proteins, rarely act alone, and usually they interact physiologically, either transiently or permanently, to assume biological functions in living systems. For example, WRKY transcription factors can interact with a variety of transcription factors to jointly regulate plant growth and development. AtWRKY50 can interact with TGA2 or TGA5 to bind to PR1 promoter and cooperate with TGA transcription factors to activate PR1 expression [226]. In addition to binding to transcription factors of other families, WRKY transcription factors can also bind to other transcription factors of their own families to play a corresponding regulatory role. WRKY transcription factors can form dimers or mulmers through protein-protein interactions before binding to DNA [227].

In addition to many of the important regulatory proteins mentioned above, there are other proteins that interact with WRKY transcription factors to play regulatory roles. VQ protein is a cofactor in plant-specific transcriptional regulation. The interaction of VQ proteins with WRKY proteins may lead to conformational changes or post-translational modifications that activate or inhibit their binding to target gene promoters [228]. In melon, a total of 24 WRKY genes were co-expressed with 11 VQ family genes [229]. Further studies revealed that Arabidopsis VQ10 and WRKY8 could form complexes in plant nuclei. The interaction between the intermediate region of WRKY8 and VQ10 promotes the DNA-binding activity of WRKY8 and positively regulates plant resistance to Botrytis cinerea [230].

Numerous WRKY-interacting proteins have been identified, and it is expected that more WRKY-interacting proteins will be identified in the future by both traditional methods (such as yeast two-hybrid) and recently developed methods (such as high-density protein microarrays) based on the identified WRKY-interacting proteins. The complex regulatory functional network of WRKY transcription factors was improved through the interaction of phase interacting proteins at different levels.

The release of genomic sequences from the pumpkin [160] has laid the foundation for molecular breeding and the exploration of useful genes for agronomical

traits. However, there is no stable and efficient genetic transformation of pumpkin presently available, which restricts the application of genetic engineering and the study of gene function. Transient expression in plants is a valuable tool for many aspects of functional genomics and promoter testing. It can be used to over-express and to silence candidate genes. It does not depend on chromosomal integration of heterologous DNA so it is a relatively facile procedure and can lead to high levels of transgene expression. Recombinant DNA can be introduced into plant cells via physical methods, via *Agrobacterium* or via viral vectors [231]. *Agrobacterium* - based transient plant transformation methods have been widely used in many plants, such as *Oryza sativa* [232], *Nicotiana benthamiana* [233], *Arabidopsis thaliana* [234, 235] and so on [236-239]. Attempts at transient transformation in pumpkin are very limited [240]. Therefore, it is significantly important to develop an efficient transient transformation system in pumpkin. Reporter genes are used to detect whether the target gene has been successfully transformed, such as *GUS*, which encodes a β-glucosidase hydrolase. It is stable and can be easily observed by histochemical staining [241].

1.4. Grafting of plants in the system of measures for growing of the *Cucurbitaceae* family and increasing their salt resistance

Against the background of the growing demand for products obtained through the cultivation of plants of the *Cucurbitaceae* family, the obstacles on the way to achieving the desired indicators regarding quantitative and qualitative yield indicators are also increasing. Among them, in particular, is the spread of diseases transmitted through the soil during the continuous cultivation of melon crops. It significantly affects the efficiency of vegetable production. At the same time, traditional measures of prevention and control often not only irrationally spend human and material resources, pollute the environment, but also do not give satisfactory results.

Grafting, which is one of the types of artificial vegetative reproduction of plants, is now considered an effective means of increasing the quantitative and qualitative indicators of the obtained products of the *Cucurbitaceae* family. Currently, grafting is actively used in the cultivation of melons, watermelons, and cucumbers. This

technology is considered as a component of measures to reduce the shortage of watermelons and melons in the non-seasonal growing period.

Grafting is an ancient skill, documented over 3000 years ago. This technology was first noted in written Chinese sources in the 16th century. Compared to fruit trees, the research and application of grafting started somewhat later for vegetables. In general, grafting became widely used in agriculture in the second half of the 19th century. It happened against the background of active scientific research in this direction all over the world. In particular, in Japan and the Korean peninsula, the large-scale application of grafting technology in vegetable production began in 1920, [242, 243], aubergines in 1950, cucumbers and tomatoes in 1960 and 1970 [244]. In Ukraine, one of the first people interested in grafting was I.M. Krajevyi (1947–1978) [245]. At present, grafting is a technology popular both in Asia and Europe. Every year, 540 million garden plants are grafted in South Korea, and 750 million in Japan [243]. In France, 2,800 hectares are under rootstock plants. In South Korea and Japan, approximately 95% of watermelons, most of the cucumbers of open soil and 30% of protected soil are grown on various grafts [245]. Considerable attention is paid to the development of this technology in China [246].

When cultivating plants of the *Cucurbitaceae* family, pumpkins are widely used grafts. The best graft among pumpkins is considered C. *moschata*, which have high adaptability, rapid seedling growth and easy propagation [247-249].

When evaluating the efficiency of the application of grafting technology, considerable attention is paid to the study of yield indicators and plant productivity. There are data that melon plants grafted onto pumpkins, compared to ingrafted ones, were larger at the initial stages of growth; they were 77.4% larger at the initial stages of growth, and 112.3% larger at the later stages [250]. In grafted melon, a higher yield of 34.3-47.3% was recorded [251]. At the same time, yield indicators depend significantly on the combination of species (cultivars, hybrids) used as rootstock and grafts [250, 252]. Indeed, grafting can achieve early harvest, extend the growing season, and increase yields, but a poor combination of plants selected for grafting can also lead to reduced yields. That is, choosing the appropriate combination of rootstock and grafts is the key for achieving high productivity of melon crops [252–256].

The change in the quality of melon vegetables after grafting is another important problem shared by producers and consumers. If the quality of melon vegetables deteriorates after grafting, then the rootstock has no consumer value. In particular, in watermelons grafted onto pumpkins, a decrease in fruit shape index, skin thickness, juice pH, and glucose content values were registered [254]. The results of evaluation of the quality of grafted and self-rooted cucumbers showed that the grafted cucumbers had a larger fruit mass. In one of the studies, it was shown that eight days after grafting, the fruit mass of grafted plants increased by 36.1–38.4%, and the length of the fruits increased by 23.7–30.2%, compared to non-grafted plants. At the same time, the content of ascorbic acid, soluble protein and free amino acids decreased gradually in the cucumbers, while the content of soluble sugar, the concentration of K and Mg increased. Other researchers reported lower vitamin C content in grafted cucumber fruits, while the protein and water content of grafted and non-grafted plants was almost the same [257, 258]. For the most part, it is noted that those changes that are registered in plant fruits when using plant grafting are not accompanied by a fundamental deterioration in the quality of the obtained products [252, 256, 259]. However, in general, in the aspect of obtaining fruits of proper quality from grafted plants, the issue of optimal selection of the combination of rootstock and grafts does not lose its relevance.

The facts recorded in grafted plants regarding yield indicators, growth rate, fruit characteristics, etc. are a natural result of physiological changes that occur during the cultivation of such plants. Scientists also pay considerable attention to this issue [255, 260, 261]. In particular, for grafted plants, the transport and distribution of ions [262], activity of enzymes and hormones [263–266], nitrogen metabolism [267, 268], productivity of photosynthesis [269, 270], functioning of the mechanism that provides resistance [271–273] are being studied.

It has been shown that compared to the root system of the graft, the root system of the rootstock is usually more developed and has a greater capacity to absorb water and nutrients [274]. Accordingly, activation of the flow of substances and changes in the concentration of nitrogen, phosphorus, calcium, magnesium, and amino acids were observed in the grafted cucumbers [275]. There is evidence that grafting in melon

vegetables contributed to increasing the efficiency of nitrogen use [276]. Against the background of grafting, there were changes in the synthesis of phytohormones, activation of energy metabolism and increase in cold resistance of plants [277]. A decrease in NH^{4+} i K^{+} content was registered in grafted watermelon seedlings. However, these plants were distinguished by higher rates of solar energy absorption, CO_2 and photosynthesis intensity in general [278]. It was found that grafted zucchini plants, compared to those rooted independently, under conditions of low temperature stress have higher stomatal conductance and initial activity of carboxylase and higher intensity of photosynthesis [279].

The greater resistance of grafted plants to diseases not transmitted through the soil is mainly due to a more developed root system, a powerful growth potential and an active flow of processes related to plant formation [280]. When studying the physiological effect of different rootstocks on grafted cucumber, it was established that against the background of grafting, the intensity of photosynthesis in plants increased by 21.81%, and at the same time, the yield also increased. Active growth and high metabolism of the root system contributed to this, which ensured the supply of a large amount of water, inorganic salts, and hormones, which had a positive effect on the growth and morphogenesis of shoot structures. In turn, the rapid growth of the shoot is accompanied by an active increase in the area of the leaves, an increase in the content of chlorophyll, and the activation of photosynthesis and synthesis of organic substances.

Experts in the field of grafting [281] believe that this technology is not just a mechanical combination of parts of different plants, but an effective interaction between them to form a single whole unit. Substances that were initially absent in the graft can be transported to it from the rootstock. In turn, the graft can also change the composition of the rootstock and thus affect its morphological and physiological characteristics. As a result, a new plant organism is formed, which differs in its characteristics from both graft and rootstock.

Therefore, grafting is also a means of influencing the eco-characteristics of individuals and modelling plants with traits that meet the demands of production. In

particular, the use of grafting is effective in solving the issue of increasing salt tolerance of members of the *Cucurbitacea*e family, which are mostly not halophytes.

Indeed, it has been proved that grafting can increase the resistance of *Cucurbitaceae* plants to salt stress [282]. In particular, on alkaline soils, the percentage of shoot mass reduction was significantly lower in plants grafted onto pumpkin rootstocks compared to non-grafted plants. Against the background of a high pH level, a decrease in the concentration of macro elements, especially P and Mg, was registered in the leaves of non-grafted plants, and in plants in general –a decrease in assimilation indicators. At the same time, grafted plants were distinguished by higher values of Fe content (on average 109.5 μg g^{-1} versus 86.7 μg g^{-1} in non-grafted plants).

The increased salt resistance of grafted plants is due to the higher stability of the membranes of the root system of the rootstock (pumpkin), the activation of the absorption of K, Ca and Mg against the background of grafting, and, as a result, the improvement of the values of the K/Na indicator, the increase in the content of saturated fatty acids in the lipid components of the membrane [283]. Damage to the plasma membrane and an increase in its permeability were registered in watermelon rootstocks against the background of salt stress. However, relative electrical conductivity was improved significantly and the degree of peroxidation of membrane lipids increased. The activity of peroxidase increased significantly, and the activity of superoxide dismutase decreased. The content of free proline increased, which is an important sign of increased salt tolerance [284]. Salinity resistance also increases when using salt-tolerant rootstocks [285, 286].

The results of many studies show that grafting, including the use of highly resistant or immune rootstocks, can improve significantly the resistance of plants to diseases (fusarium, downy mildew, gray mold, etc.) [287–290]. As a result, it is accompanied by an increase in plant survival, yield, average fruit weight and soluble dry matter content [291, 292].

Therefore, the analysis of literary sources shows that the response and adaptation of plants to salinity is accompanied by a number of physiological and biochemical processes. In the aspect of ensuring the adaptation and survival of plants in conditions of salinity, an important role, in particular, is played by the functioning of salt

tolerance genes, the formation of the antioxidant system of enzyme protection, the activation of the synthesis of proline and other adaptive substances, and the accumulation of glycine betaine. The success of finding out the mechanisms of salt tolerance is closely related to the general development of science. At the current stage, the degree of detail in the problem disclosure has significantly increased due to the use of the latest methods and technologies, including those that allow revealing the essence of adaptation processes, starting with the molecular level and the implementation of genetic control. These approaches are promising and will certainly allow us to deepen our knowledge of both the essence and the interaction of the processes that occur in plants under the influence of high concentrations of salts. An urgent issue is to find out the mechanisms of salt tolerance of representatives of the economically valuable family *Cucurbitaceae* and genus *Cucurbita*, which are not halophytes, and quite sensitively react to salt stress, however, the mechanisms of this response have not yet been sufficiently studied.

Undoubtedly, at the current stage, the use of the latest scientific approaches, in particular, genetic engineering and study of symbiotic relationships between plants, opens up wide opportunities for deepening knowledge about the formation of salt resistance in plants. However, further research on ensuring salinity of plants based on the use of traditional technologies, such as grafting, does not lose its relevance.

Despite the ancient history of its origin, in modern conditions, grafting is a technology that is not only widely used, but also is constantly improved. Its application in the system of measures for growing plants of the *Cucurbitaceae* family is an integral part of solving one of the priority problems of humanity: providing the population with food products. First, this is ensured by the fact that the use of grafting provides an opportunity to increase the resistance of plants to adverse environmental factors (for example, soil salinity, exposure to low temperatures) and diseases, and as a result, to increase their yield and production volumes. The growth of the last indicator is also facilitated by the achievement, thanks to the introduction of grafting, of the continuity of the cultivation of plants of the *Cucurbitaceae* family. Despite significant theoretical and practical developments, research on issues and problems related to grafting technology continues. Currently, considerable attention is paid to the in-depth

investigation of the physiological and biochemical aspects of the interaction of graft and rootstock, as well as the issue of achieving their optimal (for specific scientific, industrial tasks and conditions) combination.

The conducted analysis not only confirms the relevance of the study of salt resistance of plants of the family *Cucurbitaceae* and genus *Cucurbita*, but also indicates the insufficiency of a comprehensive study of this problem covering different levels of the organization.

Therefore, the purpose of our dissertation work was determined to establish of the complex mechanisms of response to salt stress and adaptation to it, which are implemented by representatives of the genus *Cucurbita* at different levels of organization, as well as eco-physiological aspects of the formation of salt resistance, based on using both classical and modern technological approaches.

The materials of the chapter are covered in four publications:

1. Хе Сунтао (2022). Фізіолого-біохімічні аспекти реагування рослин на засолення ґрунту (оглядова). Вісник Сумського національного аграрного університету. Серія: Агрономія і біологія, 50 (4), 62–68. https://doi.org/10.32845/agrobio.2022.4.9
2. Хе Сунтао (2023). Щеплення у системі заходів із вирощування рослин родини *Cucurbitaceae*. Вісник Сумського національного аграрного університету. Серія «Агрономія і біологія», 51 (1), 129-136. DOI https://doi.org/10.32782/agrobio.2023.1.15
3. **He Songtao**, Zhou Junguo, Skliar V. H. & Xinxiang (2021). Mechanism of plant adaptation to osmotic stress. «Гончарівські читання»: Матеріали Міжнародної науково-практичної конференції, присвяченої 92-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем‘яновича (25 травня 2021 р.). Суми, 2021, 108–109.
4. He Songtao (2022). Results of the analysis current status of salinization worldwide. Матеріали Всеукраїнської наукової конференції студентів і аспірантів, присвяченої Міжнародному дню студента (м. Суми, 14-18 листопада 2022 р.), 32.

CHAPTER 2
STUDY OF THE INFLUENCE OF VARIOUS CONCENTRATIONS OF SALTS AND SALT STRESS ON THE PLANTS (METHODOLOGY)

2.1. Scheme of the experiment and assessment of the effect of different concentrations of salt on the habit of plants, the ionic composition, and the degree of salt damage

An experiment on the influence of different concentrations of salts was conducted in the greenhouse of Henan Institute of Science and Technology from March to August 2020 with varieties Yanzhen and Miben. At the beginning of research fifty seeds of the same size were selected for each test material, disinfected with 0.1% $KMnO_4$, soaked at 28℃ for 8h, rinsed with sterile water for 5 times, inoculated in sterile 12 cm petri dish with 2 layers of filter paper, added with 3–5 ml sterile water, sealed with a sealing film, and then promoted germination under the condition of darkness and constant temperature (27±1 °C). After germination, the neatly sprouted seeds were planted in a nutrient bowl containing vermiculite and perlite (2:1) and planted in a greenhouse equipped with a heating system. Greenhouse management day temperature of 25 ~ 28℃, night temperature of 15 ~ 18℃, light intensity of natural light, relative humidity of 50% ~ 70%, other management is the same as the general solar greenhouse. Seedlings were transplanted when they reached two leaves and seeded in a mixture of nutrient soil and perlite (2:1). The culture container shall be a rectangular plastic culture container with a length of 60 cm, a width of 50 cm and a height of 40 cm (Fig. 2.1, Appendix A).

The seedlings were treated with 0, 60 and 120 mmol/L NaCl solution (in increments of 60 mmol/L each time). To prevent salt shock effect, the seedlings were treated three times on the 22nd, 24th and 26th respectively until the set concentration (60, 120 mmol/L) was reached.

A

B

C

D

Fig. 2.1. Pumpkin plants at different stages of research

During the whole experiment, the water content of the soil was kept at 15%~16%, and the water was replenished quantitatively with a watering can.

2 cultivars and 2 NaCl treatments (60 and 120 mmol/L) were used. Each treatment was repeated 3 times, with no NaCl treatment as the control.

Foremost, the influence of salinity on the morphometric parameters of plants was studied. At the same time, they relied on the experience accumulated by Sumy Scientific School of Population Ecology of Plants regarding the methodology and rules of morphometric analysis [293, 294]. In the process of the experiment, static metric indicators such as stem diameter, stem length, and leaf area were primarily evaluated (Appendix B). Based on the results of morphometric analysis, according to the methodology of Yu.A. Zlobin, the vitality of plants was assessed, as well as the ratio of the proportion of individuals of different levels of vitality (the highest – class «a», the intermediate – class «b», the lowest – class «c») among the studied groups of plants [294–296].

Stem diameter, stem length and leaf area of the seventh tubercle were measured when the plant was grown (May 24). Results After harvest (July 10), the whole plant was washed with deionized water, and each plant was divided into four parts: root, stem, leaf and fruit. The plant was degreenized at 105 ℃ for 15 minutes, and dried at 65 ℃ to constant weight, which was called dry weight.

Salt damage index is an important index reflecting the damage degree of salt stress on plants. Salt damage index and salt damage rate [297]: all plants were observed and counted to calculate the damage index and damage rate of each plant under each treatment. The classification standard of salt damage was 0: no symptoms of salt damage; Grade 1: mild salt damage, with a small part (about 1/5) of leaf tips and leaf edges yellowing; Grade 2: moderate salt damage, with about 1/2 of leaf tips and leaf edges yellowing; Grade 3: moderate salt damage, most leaf tips and leaf edges burnt yellow. Grade 4: extremely severe damage, leaf scorched, fell off, withered branches, and eventually died. The formula for calculating salt damage index and salt damage rate is as follows:

$$\text{Salt damage index (\%)} = \frac{\sum\left(\text{Level of salt damage} - \frac{\text{Corresponding series}}{\text{Grade plant number}}\right)}{\text{Total plant number} - \text{Maximum salt damage value}}$$

$$\text{Salt damage rate (\%)} = \frac{\text{The numbe r of plants affected by salt appeared}}{\text{Total plant number}}$$

Characteristics of ion uptake and accumulation in Chinese pumpkin plants under NaCl stress

It is an important content of crop cultivation research under salinization and secondary salinization condition to study the mechanism of plant salt damage and salt tolerance and to select salt tolerant varieties. The key to solve this problem is to adopt salt-tolerant rootstocks for grafting and cultivation. Pumpkin is the main rootstock in the production of melons, but the salt tolerance of different pumpkin rootstocks in the production is quite different.

In this research, the salt-tolerant material 'salt stock' and the commercial variety Milbon selected by us from Chinese pumpkin (*C. moscchata* Duch.). The state of the pot culture under conditions of stress created by NaCl was studied.

At present, the commonly used pretreatment methods related to element content detection include dry digestion, wet digestion, microwave digestion and pressure tank digestion, etc. [298–300]. Compared with other methods, microwave digestion has the characteristics of strong capacity, high efficiency, less solvent consumption and low blank space, and can effectively control sample volatilization loss and reduce the risk of sample contamination. It has improved the accuracy and precision of analysis and is widely used in the pre-processing of metal element analysis in food [301].

There are many kinds of technologies for detecting and analysing the content of metal elements in substances, the main ones are: atomic fluorescence spectrometry [302, 303], flame atomic absorption spectrometry (FAAS) [304, 305], Graphite Furnace Atomic Absorption spectrometry (GFAAS) [306], Inductively coupled Plasma Emission spectrometry (ICP-AES) [307] and inductively coupled Plasma Mass spectrometry (ICP-MS) [308–310], etc. The materials used in the first three spectroscopic methods are specially manufactured hollow cathode lamps, which can

only analyse and detect one metal element in the sample each time, which not only greatly limits the types of elements measured, but also increases the measurement cost due to the extremely high price of hollow cathode lamps, which also cannot meet the tester's requirements for experimental data analysis. ICP-AES is a spectral analysis technique using inductively coupled plasma torch as excitation light source. It can determine various metal elements in samples very quickly, easily and accurately, and there is no obvious matrix effect. Up to now, ICP-AES analysis technology has become one of the most important methods in modern detection and analysis technology.

Compared with the above analysis methods, ICP-AES analysis has the following advantages: The analysis is rapid. ICP-AES technology has low interference, relatively stable spectral distribution time will not change with time, wide linear range is easy to observe, and can identify and detect the characteristic spectrum of a variety of sample elements and can simultaneously conduct quantitative and qualitative analysis and detection of a variety of metal elements. High sensitivity. It can be measured directly without any other operation, and the concentration has little effect on the detection, even a small concentration can be detected. High accuracy. ICP-AES is one of the least interferential analytical techniques and methods. The relative standard deviation is less than 10% under normal conditions, and as low as 1% when the concentration of analyte is more than 100 times the detection limit. A wide range of detection and analysis. It can measure and analyse several or even dozens of metal elements at a time.

Therefore, the microwave digestion-ICP-AES method was used to quantitatively analyse the trace and trace metal elements in pumpkin. Meanwhile, the electrothermal plate digestion samples were pre-processed. At the same time, the inductively coupled plasma mass spectrometry was carried out in-depth discussion and research, so that the optimized method and setting could be applied to the analysis of metal elements in pumpkin.

Instruments and reagents

Optima 2100DV inductively Coupled Plasma Emission Spectrometer, PE, USA; XA-2 mill, Jintan Chengdong Xinrui Instrument Factory; ML-2-4B temperature regulating electric heating plate, Jintan Huaou Experimental Instrument Factory; MDS-6G microwave digestion and extraction technology was used for sample digestion. Shanghai Xinyi Weibo Chemical Technology Co., LTD. Its main parameters are shown in Table 2.1.

Table 2.1. Main parameters of microwave digestion instrument

Content	Range of values
Power supply voltage	～220V(± 10%)50Hz
Rated power rating	1600W
Rated microwave output power	0～1000W
Microwave frequency	2450MHz±50MHz

Sample pretreatment and solution configuration

Sample the dried samples in 2.1, 3 plants per treatment, according to the root, stem, leaf, fruit, mixed, crushed and mixed with high-speed grinder. The treated samples of 0.50 g (accurate to 0.001g) were accurately weighed respectively and placed in microwave digestion tank (PTFE), then 10 mL concentrated nitric acid was added, 2 mL 30% hydrogen peroxide (H_2O_2) was poured into PTFE container, placed on the rotating turntable of microwave oven, pre-digestion at 120 ℃ for 45 min. Then, digestion was carried out according to the working parameters of the microwave digestion instrument in Table 2.2. After digestion, the acid was driven to 0.5-1ml at constant temperature on the electric heating plate at 160 ℃, and the acid driven solution was put into a 25mL volumetric bottle with 3% HNO_3 for constant volume testing. The specific measurement conditions are shown in Table 2.3. At the same time, standard quality control samples, parallel samples, reagent blank and other quality control measures are made.

Table 2.2. Operating conditions of microwave digestion instrument

Steps	1	2
T（℃）	140	180
Power（W）	600	600
Time（min）	10	10

Table 2.3. Working conditions of ICP-AES (Optima 2100 DV)

Content	Technical parameters
Radio frequency power	1300 W
Atomizer flow rate	0.2 L/min
Plasma cooling gas flow rate	15 L/min
Auxiliary gas flow rate	0.2 L/min
Peristaltic pump sampling quantity	1.50 mL/min

Drawing of standard curve

Dilute the mixed standard solution with 2% HNO_3 solution in A certain proportion to obtain the corresponding standard solution: 0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L, 5 mg/L. Absorbance A is chosen as the vertical coordinate, and the horizontal coordinate represents the concentration C of each element in standard solution. Standard curve drawing is done by instrument.

2.2. Effect of pumpkin rootstock on the growth of grafted watermelon

Grafting cultivation is the main form in the production of melon vegetables at present. Watermelon (*Citrullus lanatus*) is one of the main fruit and vegetable horticultural crops and one of the 20 most important crops worldwide. China is the world's largest country in watermelon production and consumption, and watermelon industry occupies an important position in China's agricultural industry.

China is rich in pumpkin germplasm resources, but there are relatively few pumpkin rootstocks suitable for watermelon grafting in the market at present, which cannot fully meet the needs of watermelon industry. Therefore, this study evaluated the effect of Chinese pumpkin Yanzhen on grafting of watermelon with different ploidy, to obtain a new combination of pumpkin stock with good grafting affinity and little negative impact on watermelon fruit quality.

In this experiment, a commercial pumpkin rootstock cultivar “Baimi112” (*Cucurbita maxima*, Henan Institute of Science and Technology), commercial triploid watermelon cultivar “Zhengzhou No.3 (3X)” and corresponding diploid watermelon line “Zhengzhou No.3 (2X)” [*Citrullus lanatus* (Thunb.) Matsum. and Nakai., Chinese

Academy of Agricultural Sciences] were used. The triploid watermelon cultivar "Zhengzhou No.3 (3X)" was obtained by crossing diploid "Zhengzhou No.3 (2X)" and corresponding autotetraploid "Zhengzhou No.3 (4X)" parental lines [311].

The seeds of pumpkin, diploid and triploid watermelon were surface sterilized with 3 % (v/v) sodium hypochlorite for 2 min followed by three washes with sterile deionized water. After germination at 30 ℃ for 24 h, the seeds were sown in 50-cell plug trays filled with a mixture (1:1:1) of peat, perlite, and vermiculite (v/v) in the greenhouse at Henan Institute of Science and Technology, with a 14/10 h day/night photoperiod at temperatures ranging between approximately 22 and 30 ℃ and ambient relative humidity. The rootstock seeds of pumpkin were sown earlier 5 days than the watermelon scion (triploid and diploid watermelon) seeds sowing. Two grafting combinations of watermelon lines were used, that is, triploid watermelon (3X) grafted onto pumpkin (3X/P) and diploid watermelon (2X) grafted onto pumpkin (2X/P). Once the pumpkin rootstock seedling produced the second and first true leaf, grafting was performed by using hole-insertion grafting method as described by Hassell et al. (2008) [312]. The ingrafted diploid and triploid watermelon lines were used as control. In order to maintain high humidity, seedlings were covered with a layer of transparent plastic film, and seedlings were placed in shade for 72 h. The plastic film was removed for a short time during initial days to control relative humidity, and it was completely removed after 10 d of grafting. When the third true leaf emerged, the own-root watermelon, grafted-root watermelon seedlings were transplanted into an open field in Xinxiang, China (35°18' N, 113°52' E) and grown under the same conditions in early May. Each line comprised of 2 rows, and each row was for 10 individuals; the spacing between the rows was 180 cm, and the spacing between individuals in a row was 50 cm. The treatment was replicated four times and was arranged in a randomized complete block design. The flowers were hand-pollinated and tagged. Five individual fruits were chosen randomly at 10, 20, 30 days, respectively, after pollination and used for testing fruit mass, dry matter content (whole fruit), sugar content (flesh) and assaying enzyme activity (flesh). The flesh (central portion) samples were collected and divided into two subsets. One subset was freeze-dried to a powder for sugar content determinations. The other subset was immediately frozen in liquid nitrogen

and stored at -80 ℃ for the enzyme assays. Each point therefore represents the average of five samples from individual fruit.

Study of pumpkin rootstock on the growth of grafted watermelon was supplemented by an assessment of the effect of mycorrhiza on the condition of plants. In this experiment, the commercial pumpkin rootstock cultivar ‘Baimi112’ (*Cucurbita maxima*), the commercial triploid watermelon cultivar ‘Zheng No.3 (3X)’ and the corresponding diploid watermelon line ‘Zheng No.3 (2X)’ [*Citrullus lanatus* (Thunb.) Matsum. and Nakai.] were used. The arbuscular mycorrhizal fungi (AMF): *Rhizophagus intraradices*, was provided by Henan Agricultural University, Zhengzhou, People’s Republic of China. Which was multiplied in a pot culture for 15 weeks in greenhouse conditions using maize. The average colonisation of Rhizophagus intraradices was 95.6% and the average spore density was 549 per 10 g of air-dried soil. The spores, mycelium, colonised root fragments, and dried sand-soil were mixed to use as AMF inoculums. The mycorrhizal inoculums were placed 15 cm below the seedlings at the time of transplanting (15 g per plant).

The treatments were: (1) well-watered and non-AMF (WW); (2) well-watered and inoculated with AMF (WW + AMF); (3) deficit irrigation and non-AMF (DI); (4) deficit irrigation and inoculated with AFM (DI + AMF). The DI treatments started five days af ter pollination and were imposed by withholding water from the plots until the soil water potential was achieved. At the same time, the well-watered plots were controlled with 80% of the field capacity (–0.075 MPa), and the DI plots were controlled with 60% of the field capacity (–0.14 MPa). The soil water potential was measured by a pressure plate apparatus (Shimadzu, CL–800, Kyoto, Japan) and the amount of water loss was supplied to each plot to keep the intended soil water contents [312].

2.3. Physiological and biochemical aspects of response to salt stress and adaptation to it

Establishing the general features and patterns of response and adaptation of pumpkins to salt stress and establishing the features and patterns of response and adaptation to salt stress of plants created during the application of grafting technology,

was accompanied by the conduct of physiological and biochemical studies, the methodology of which is outlined in this subsection.

The net photosynthetic rate (PN)

PN: Net photosynthetic rate was measured under the conditions of natural environment (field). We also recorded the change process of photo flux density (PFD) and temperature in experimental location at this time (Fig.2.2). LI6400 portable photosynthesis system (LI-COR co., USA) was used to measure the PN of the third leaf (from the top) at 10, 20, 30 days, respectively, after pollination under natural conditions. They were measured at 8:00, 9:00, 10:00, 11:00, 12:00, 13:00, 14:00, 15:00, 16:00, 17:00, 18:00 in a day. Each result shown was the mean of 10 replicated treatments.

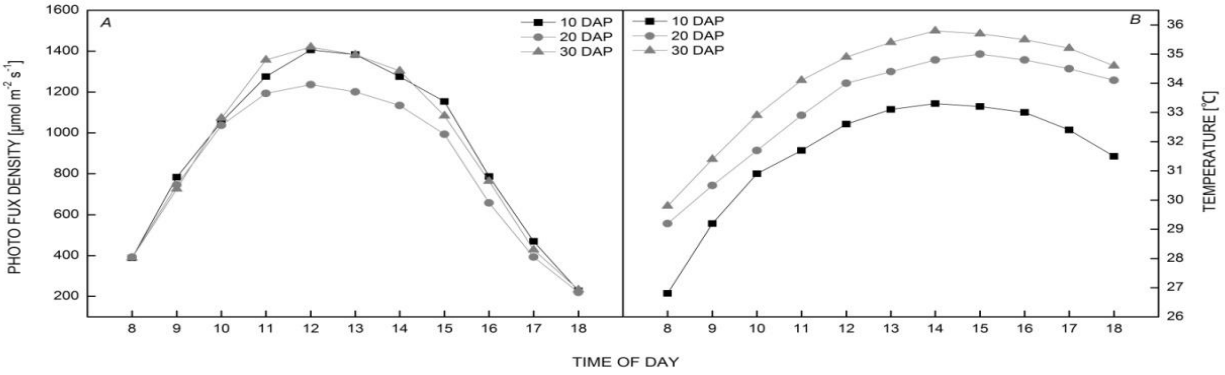


Fig. 2.2. Diurnal changes of light intensity (A) and temperature (B) at 10, 20, 30 d after pollination. DAP – days after pollination

Chl fluorescence parameters

The Chl fluorescence parameters of the third leaf were measured with a portable chlorophyll fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). The mean values of leaf electron transport rate (ETR), maximum photochemical efficiency of PSII (Fv/Fm) and actual photochemical efficiency of PSII (ΦPSII) were measured as described by Baker (2008) [314]. Each result shown was the mean of 10 replicated treatments.

Sucrose and total sugar determination

Sucrose and total sugar content was assayed as described by Liu et al. (2013) [315]. Approximately 200 mg of freeze-dried flesh per sample was ground to a fine powder and extracted for 1 h in 10 mL of 50 % ethanol at 80 ℃ and then centrifuged at 3,000 g for 10 min. The pellet was again extracted and centrifuged, and the combined supernatants were placed in a volumetric flask (25 mL); 2 mL of the sample was centrifuged at 3,000 g for 10 min. The supernatants (1 mL) were filtered through a 0.45-lm HPLC nylon filter (Membrana, Germany). The sugars in the sample were separated in an analytical HPLC system (Pump System LC-10ATVP, Shimadzu, Japan) fitted with a Shodex Asahipak NH2P-504E column (4.6 × 250 mm; Shodex, Japan) using a refractive index detector (RID-10A, Shimadzu, Japan).

Extraction and assay of alkaline α-galactosidase

The alkaline α-galactosidase activity was assayed according to the method of Gao and Schaffer (1999) [316], with some modifications. Approximately 1 g of freshly frozen flesh was homogenized in a mortar with four volumes of extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 2 mM EDTA and 5 mM DTT. All samples were centrifuged at 18,000 g for 20 min at 4 ℃ to separate the supernatants. After centrifugation, the supernatants were collected for alkaline α-galactosidase analysis using p-nitrophenyl-a-D-galacopyr-anoside (pNPG) as a substrate. The initial reaction buffer contained 5 mM pNPG in 50 mM Hepes buffer (pH 7.5). The samples were incubated at 37 ℃. The reaction was terminated after 10 min by adding four volumes of 0.2 M Na_2CO_3. The release of p-nitrophenol was measured spectrophotometrically at 410 nm, and p-nitrophenol (Sigma) was used as a standard.

Insoluble acid invertase (IAI) extraction and assay

Insoluble acid invertase activity was measured according to the method of Miron and Schaffer (1991) [317], with some modifications. Approximately 1 g of freshly frozen flesh was homogenized in a mortar with three volumes of extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 0.5 mM Na-EDTA, 2.5 mM DTT, 3 mM diethyldithiocarbamic acid, 0.5 % (w/v) BSA and 1 % (w/v) insoluble

polyvinylpyrrolidone (PVP). All samples were centrifuged at 18,000 g for 30 min at 4 ℃ to collect the insoluble pellet (containing crude insoluble acid invertases) by separation. The insoluble pellet was homogenized in the extraction buffer, centrifuged and suspended in 3 mL of 50 mM Hepes-NaOH (pH 7.5) and 0.5 mM Na-EDTA. To solubilize the "insoluble" acid invertase enzyme, NaCl (0.5 M final concentration) was added to the initial extraction buffer prior to extraction. All samples were incubated at 4 ℃ for 10 min and centrifuged at 18,000 g for 20 min at 4℃. The supernatants were collected for analysis of crude insoluble acid invertase activity. Insoluble acid invertase activity was assayed in 0.8 mL of 0.1 M K_2HPO_4 - 0.1 M citrate buffer (pH 5.0), 0.2 mL 0.1 M sucrose and 0.2 mL of enzyme extract (for the control). All samples were incubated for 30 min at 37 ℃, after which the reactions were stopped at 100℃ for 5 min. After cooling, colour development was measured at 540 nm. Enzyme was added to one sample after the 30-min incubation for the blank control.

Extraction and assay of SuSy and SPS

SuSy and SPS were extracted according to the methods of Hubbard et al. (1989) [318] and Lowell et al. (1989) [319], with some modifications. Frozen flesh was homogenized in a chilled mortar using a 1:5 tissue-to-buffer ratio. The buffer contained 100 mM phosphate buffer (pH 7.5), 5 mM MgCl2, 1 mM EDTA, 2.5 mM DTT, 0.1 % (v/v) Triton X-100 and 2 % PVPP (w/v). The homogenates were centrifuged at 10,000 g for 30 min at 4 ℃. After centrifugation, the supernatants were dialysed in a 15-cm dialysis tube (MwCO:8,000–15,000) for approximately 16 h at 4 ℃ against a solution containing 10 mM phosphate buffer (pH 7.5), 0.5 mM MgCl2, 0.1 mm EDTA, 0.25 mM DTT and 0.01 % (v/v) Triton X-100. The solution remaining in the dialysis tube was collected for analysis of crude SuSy and SPS activity. SPS activity was assayed by adding 0.1 mL of crude extract to 50 μL Hepes buffer [50 mM Hepes–NaOH (pH 7.5), 15 mM $MgCl_2$, 15 mM fructose-6-PNa2, 15 mM glucose-6-PNa2 and 15 mM UDP-glucose]. The samples were incubated for 30 min at 37 ℃, after which the reaction was stopped by the addition of 0.2 mL 30 % (v/v) KOH. The tubes were placed in boiling water for 10 min to destroy any non-reacted fructose or fructose-6-P. After cooling, 3 ml of a mixture of 0.14 % (w/v) anthrone in 13.8 M

H_2SO_4 was added to each sample, and the samples were incubated in a 40°C-water bath for 20 min. After cooling, colour development was measured at 620 nm. The procedure for the SuSy assay (measured in the sucrose synthesis direction) was identical to that of SPS except that the reaction mixtures contained 0.1 M phosphate buffer (pH 8.0) and 60 mM fructose and did not contain fructose-6-P or glucose-6-P.

2.4. Genetic aspects of response to salt stress and adaptation to it

2.4.1. Identification and expression analysis of WRKY gene family in pumpkin under salt stress

After a long period of evolution, plants have formed a series of mechanisms for regulating their growth and development and resisting biotic and abiotic stress and adversity. WRKY transcription factors are the largest family of transcription factors in higher plants and widely exist in the entire plant lineage [320].

Acquisition and identification of pumpkin WRKY family members

Physiological and biochemical ownload the pumpkin (*Cucurbita*) protein group and genome data melon/v3.6.1 (ftp://cucurbitgenomics.org/pub/cucurbit/genome/), Blast localization software (Blast2.2.28) was used to construct a local protein and gene database. Download the hidden Markovmodel (HMM) file of WRKY conservative domain (serial number PF03106) from the Pfam database (http:// pfam.xfam.org/) (El-Gebali et al., 2019) [321] and use it as the search sequence. HMMER3.2 software [322] was used to conduct BlastP search in pumpkin local database, and the threshold was set as E-value < 10-10 to obtain pumpkin WRKY candidate proteins. Candidate protein sequences were predicted by SMART (http://smart. embl. de/) online software to further identify whether pumpkin WRKY candidate proteins contain WRKY domains, using ProtParam (http:// web.expasy.org/protparam/) analysis of pumpkins member of the WRKY protein sequence length, molecular weight and isoelectric point. Euk - mP - Loc2.0 subcellular localization analysis (http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/) [323].

Chromosome localization, evolutionary tree and conserved motif analysis

When finding the location of the CmWRKYs chromosome in the pumpkin gene database (http://cucurbitgenomics. org/organism/18), map the chromosome distribution of CmWRKYs using MapChart 2.2. The gene and protein sequences of Arabidopsis WRKY members (Eulgem et al., 2000) [324] were derived from the Arabidopsis database (http://www.arabidop-sis.org/). MEGA X10.0.5 [325] was used to construct the WRKY phylogenetic tree of *Arabidopsis thaliana* and pumpkin. The sequence alignment method was MUSCLE and neighbour-joining (NJ) method was used to construct the phylogenetic tree. The execution parameters are p-distance and pairwise deletion, respectively, and the number of repetitions of bootstrap is set to 1000 [326-328], and select the default option for the remaining Settings. According to the division method of Arabidopsis WRKY family members, the pumpkin WRKY family members were classified, and the results were shown by Evolview2 [329]. MEME5.1.0 software was used to analyze the conserved motifs of CmWRKY protein, and the parameters were set as the minimum motif length was 15aa, the maximum motif length was 150aa and the maximum number of conserved sequences was 20 [330].

Pumpkin material culture and salt treatment

The pumpkin was a hybrid variety 'Salt Anvil' bred by Henan Institute of Science and Technology. The seeds were disinfected with 5% sodium hypochlorite solution for 10 minutes, then cleaned with distilled water for 3 times, and then soaked in distilled water for 8h, the seeds were sown in the bud stimulating dish, and the buds were stimulated at 26℃ for 3 days. Seeds with uniform germination were selected and seeded in nutrient bowls containing matrix (vermiculite ： nutrient soil =3:1), with 1 seed in each bowl, a total of 160 seeds. Then it was cultured in a light incubator with a photoperiorcycle of 16h light/8h darkness, an average light intensity of 6000lx, and a day/night temperature of 26℃/24℃. Salt treatment was carried out in the four leaves of pumpkin at the same time. The day/night temperature was set as 9℃/6℃, and the day/night temperature of 26℃/24℃ was used as the control. The photoperiod and light intensity were the same as above. Pumpkin leaves were completely unfolded at 0,

12 and 24 hours of treatment, respectively. The leaves were repeated 3 times at each time point, and 5 mixed plants were taken each time. The samples were quick-frozen in liquid nitrogen and stored at -80℃ in the refrigerator.

qRT-PCR analysis of CmWRKYs gene expression

Total RNA from pumpkin leaves was extracted by Trizol reagent (TIANGEN, Beijing). cDNA was synthesized by reverse transcription kit (TaKaRa, Dalian). According to the results of CmWRKYs gene evolution, some homologous WRKY genes in each subgroup were selected for salt stress induction analysis. CmWRKYs and its internal reference gene (β-actin, GeneBankNo. AY859055) specific primers were designed with PrimerPrimer5.0. The primer was synthesized by Shanghai Sangon Biological Co., LTD. PCR reaction system: 10μL SYBR Green Mastermix (2×), 10μmol/L upstream and downstream primers each 0.8μL, cDNA template 2μL, ddH2O6.4μL. PCR reaction procedure: predenaturation at 95℃ for 30 s; 95℃ 5 s, 60℃ 30 s, 50 cycles. With the expression level of 0h control group as the total control, the relative gene expression level of control group and treatment group at 12 and 24h was calculated by 2-ΔΔCt method (Livak, Schmittgen, 2001) [290], and the calculated results were converted by log2 (relative expression level +1) formula. The pheat map package in R-3.6.0 software was used to map the qRT-PCR results of 26 genes. For 11 of the genes that were highly up-regulated, Levene test of variance, Analysis of variance (aov function), Tur- key's honestly significant difference (HSD) and Fish- in R-3.6.0 software were used Er 'sleast significant difference to analyse significant difference (LSD)) and marking, the histogram display using ggplot2 package.

2.4.2. Transient transformation system for gene function studies in pumpkin

The release of genomic sequences from the pumpkin [160] has laid the foundation for molecular breeding and the exploration of useful genes for agronomical traits, including to achieve increased salt resistance. Recombinant DNA can be introduced into plant cells via physical methods, via Agrobacterium or via viral vectors [231].

Currently, gene exploration and molecular breeding in pumpkin (*Cucurbita moschata* Duch.) is a challenging process. There are limited efficient stable

transformation methods for pumpkin [240]. Therefore, it is significantly important to develop an efficient transient transformation system in pumpkin.

Transient transformation is a promising tool for the study of gene function. Here, an efficient Agrobacterium-mediated transient transformation system was developed for gene function studies in pumpkin. In this study, we also investigated the effect of sonication and vacuum infiltration on transformation efficiency via the GUS reporter gene.

The objects of research were pumpkin inbred lines coded ‘360− 3’, ‘112− 2’, ‘009 − 1’, ‘042 − 2’, ‘Chang – 2’ and ‘Jiujiang’ were used as plant materials for the experiments. All these pumpkin resources were obtained from our pumpkin breeding and research team.

Research was carried out in several stages [331]:

1. Sonication and vacuum infiltration
2. GUS assay
3. Salt stress treatment and determination of physiological indicators
4. Cotyledon injection
5. GFP fluorescent detection
6. Vector construction and Agrobacterium transformation

As a result, the vector pBI121-EGFP was isolated and digested with Xba I and Sma I (N-terminus of EGFP), and then purified by gel electrophoresis.

Primers were designed according to full-length cDNA with no stop codon of CmoCh05G005550 (named as CmPHD1, no introns, 717bp) released from the Cucurbit Genomics Database (http://cucurbitgenomics.org/).

Forward primer: 5′ -GCTCTAGAATGGATTTCGATGAGGAGCAC-3′

Reverse primer: 5′ -TACCCCGGGATCATTTGAGCACTTCTTC-3′

The underlined sequences are of Xba I and Sma I cutting sites, respectively.

The pumpkin genome DNA was isolated and used as a template for PCR.

The T4 DNA ligase (Takara, Dalian, China) was used to ligate the fragment and vector (16 ◦C, 1 h). The 10 μL ligation liquid was transformed into E. coli (DH5α) competent cells via a heat shock method. The recombinant vector, pBI121-CmPHD1-EGFP, was isolated and stored at − 20 ◦C for further use. Agrobacterium tumefaciens

EHA105 competent cell was purchased from a company (Biomed, Beijing, China). The new construct was transformed into Agrobacterium based on the instructions from the manufacturer.

Mathematical statistics methods and appropriate software were used to summarize the data collected during the research. It was widely used SAS software (SAS Institute, Inc., Cary, NC, USA). In particular, they calculated means ± SE. Used one-way and two-way analysis of variance (ANOVA /ANOVA), method (Tukey's multiple range test) [332].

The use of the set of methods, described in Chapter 2, made it possible to comprehensively (at the level of individuals, physiological and biochemical changes, functioning of genetic mechanisms) study and analyse the adaptation, response of plants of the *Cucurbitaceae* family to salt stress, identifying possible means of regulating their salt resistance (based on the use of both classic technologies (grafting) and the latest (genetic engineering etc.)).

The materials of this chapter are mainly covered in three scientific publications, which were published in publications entered scientific databases (Scopus, Web of Science):

Xuejin Chena, **Songtao He**, Lina Jiang, Xinzheng Li, Weili Guo, Bihua Chena, Junguo Zhoua &Viktoriia Skliar (2021). An efficient transient transformation system for gene function studies in pumpkin (*Cucurbita moschata* D.). Scientia Horticulturae, 1, 1-10. DOI:10.1016/j.scienta.2021.110028

Yang, P.M., **He, S.T.,** Jiang, L.N., Chen, X.J., Li. Y.F. & Zhou, J.G. (2020). The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose content of different ploidy watermelon (*Citrullus lanatus*). Photosynthetica. 58 (5), 1150-1159. DOI: 10.32615/ps.2020.068

Yang, P.M. & **He, S.T.** (2022). The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*). Hort. Sci. (Prague), 49, 225–233. https://doi.org/10.17221/108/2021-HORTSCI

CHAPTER 3
RESPONSE AND ADAPTATION OF PUMPKIN PLANTS TO SALT STRESS (RESULTS)

3.1. The influence of different concentrations of salts on morphological characteristics, viability and degree of damage to pumpkin plants

3.1.1. The effect of salts on the on morphological signs and vitality of pumpkin plants

At the beginning of the research, the studied plants at the beginning of the research did not have statistically significant differences in the values of morphoparameters (Table 3.1).

Table 3.1. Dimensional indicators of pumpkin plants at the beginning of research

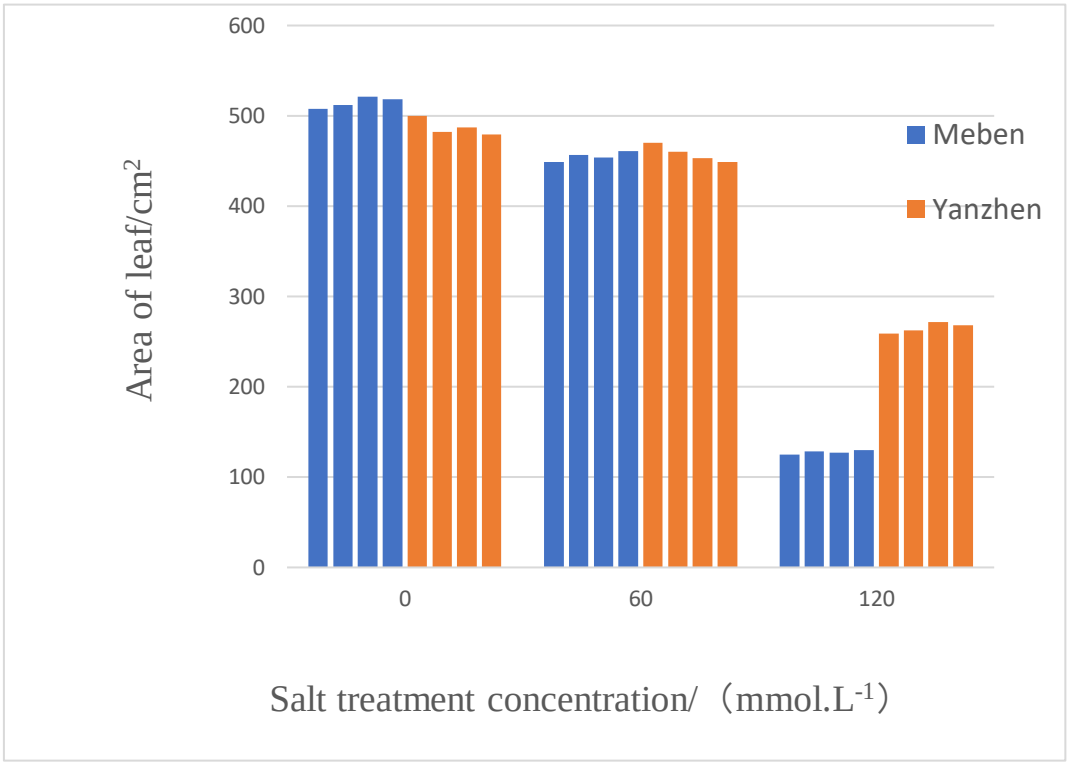
NaCl concentration ($mmol.L^{-1}$)	Dry weight	Leaf area	Stem length	Stem thickness
0	1.0	1.0	1.0	1.0
60	1.0	0.92	0.84	1.0
120	1.0	1.0	1.0	0.89

After NaCl treatment, the leaves and plant height of pumpkin plants became smaller. With the increase of salt concentration, the inhibition degree of plants increased gradually. After 60 mmol/L NaCl treatment, the growth of the plants was delayed, some tips and margins of Miben were yellowish, and the leaves of Yanzhen were slightly smaller. At 120 mmol/L, the plant growth was further delayed, and most of the leaf tips and margins of Miben showed obvious symptoms of salt damage, severely abnormal growth, severe leaf curl, lodging and even death, and even could not flower and bear fruit normally. The Yanzhen leaves are further reduced, with some tips and margins browned, but can flower and bear fruit normally (Fig. 3.1, Appendix A).

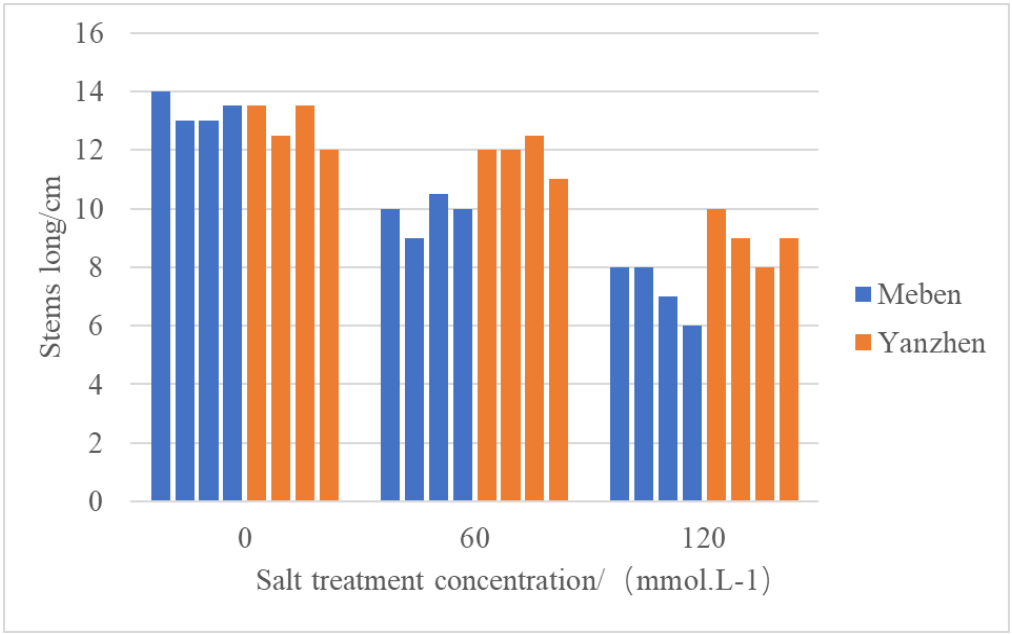
Fig. 3.1 Pumpkin plants treated of NaCl

After NaCl treatment, stem diameter, stem length, leaf area and dry weight were significantly affected (Figure 3.2, Appendix B). After treatment with 60 mmol/L NaCl, all the characters of NaCl sensitive Miben showed significant decrease. However, the Yanzhen with less NaCl sensitivity only showed a decrease in dry weight. After 120 mmol/L NaCl treatment, leaf area and dry weight were significantly affected, followed by stem length, and stem diameter was least affected.

A


600
500
400
300
200
100
0
Area of leaf/cm2
Meben
Yanzhen
0
60
120
Salt treatment concentration/（mmol.L-1）

B


16
14
12
10
8
6
4
2
0
Stems long/cm
Meben
Yanzhen
0
60
120
Salt treatment concentration/（mmol.L-1）

C

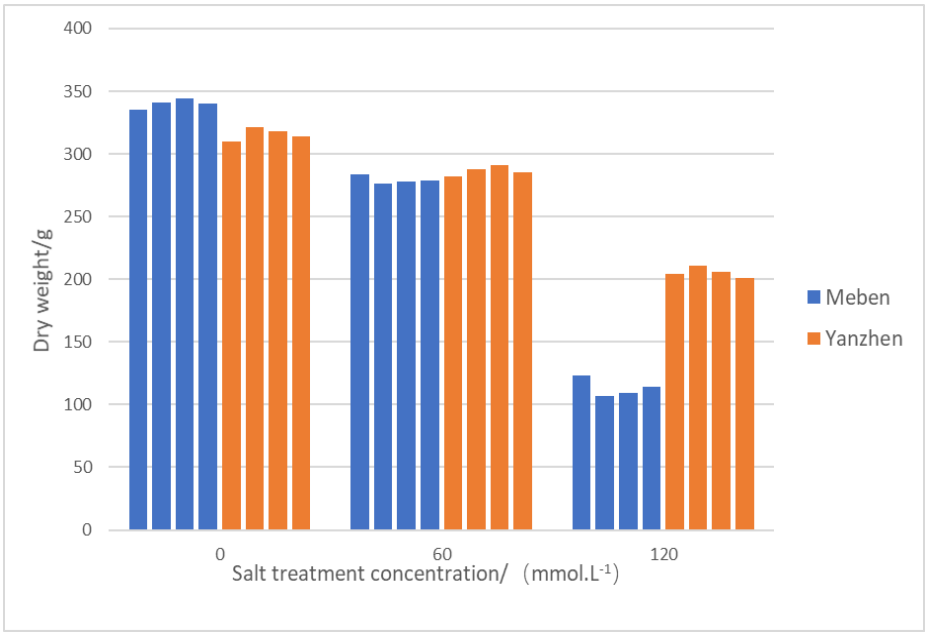


Fig. 3.2. Effects of different concentrations of NaCl on pumpkin plant traits

Among them, the stem diameter, stem length, leaf area and dry weight of Miben were lower than those of Yanzhen. In general, Miben was the most sensitive to salt stress, and its growth and development were affected the most. The Yanzhen was the least sensitive to salt stress, and the growth and development were the least affected.

The results of the vitality analysis based on these morphometrics (Table 3.2) showed that, against the background of an increase in the concentration of salts among pumpkin plants, the proportion of individuals with the highest vitality (class «a» vitality) decreases from 100 to 58% the variety Miben and from 100% to 75% the variety Yanzhen, and the proportion of plants increases of lower vitality (class «b»): from 0% to 42% the variety Miben and from 0% to 25% the variety Yanzhen. The results of the vitality analysis also confirmed the higher sensitivity of plants of the variety Miben to the influence of increased salt concentrations.

Table 3.2. Vitality of pumpkin plants against the background of different concentrations of salts

№	Variety	Nacl/ (mmol/L)	The share of plants of different classes of vitality		
			a	b	c
1	Miben	0	1,0	0	0
2		60	0,92	0,08	0
3		120	0,58	0,42	0
4	Yanzhen	0	1,0	0	0
5		60	0,83	0,17	0
6		120	0,75	0,25	0

3.1.2. Influence of different concentrations of NaCl on salt damage index of pumpkin

Indicators of salt damage of pumpkin treated with different concentrations of salt are shown in Table 3.3. As can be seen from Table 3.3, with the increase of NaCl concentration, the salt damage index and salt damage rate of the two kinds of pumpkin showed an increasing trend. When the concentration of NaCl reached 60mmol/L, the salt damage symptom of Miben was obvious, the salt damage index and salt damage rate reached 19% and 33.3%, respectively. But Yanzhen did not show obvious symptoms of salt damage, salt damage index and salt damage rate are significantly lower than Miben.

When the concentration of NaCl reached 120 mmol/L, the salt damage index of Miben reached 37%, and the salt damage rate reached 100%.

Table 3.3. Salt damage index and salt damage rate of two kinds of pumpkin varieties treated with different concentrations of NaCl

NaCl/ (mmol/L)	Salt damage index, %		
	Miben	Yanzhen	F-value[1]
0	0.00	0.00	0
60	19	0	1.87
120	37	18	2.42
	Salt damage rate, %		
	Miben	Yanzhen	F-value
0	0.00	0.00	0
60	33.33	0.00	1.77
120	100	41.67	4.20

Note: $F_{0.05} = 5.14$; $F_{0.01} = 10.92$

While the salt damage index of Yanzhen is 18%, the salt damage rate is only 41.67%, the increase range is obviously less than Miben. These results indicated that the two kinds of pumpkin had significantly different salt tolerance under NaCl stress. Yanzhen showed the latest symptom of salt damage, the salt damage index and salt damage rate were significantly lower than Miben, showing a strong salt tolerance. However, Miben showed early symptoms of salt damage, the salt damage index was significantly higher than Yanzhen, and the salt tolerance was weak, so it was a salt sensitive variety. Both the salt damage index and salt damage rate reflect the salt resistance ability of plants under different concentrations. Therefore, when the two indexes are combined to reflect the damage to plants under salt stress, the results will be more objective. After comprehensive analysis, it can be seen that Miben had the worst tolerance and Yanzhen had strong salt resistance of pumpkin from two different provenances.

3.2. The influence of salt stress on the exchange of organic compounds and leading physiological processes

3.2.1. The influence of salt stress on indicators related to the flow of photosynthesis and water exchange of pumpkin.

Net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) were measured by LI-6400XT portable photosynthesometry system at 9：00 ~ 11：30 in the morning. An open gas path was used for measurement. CO_2 was collected from the relatively stable 3~4m high air outside the greenhouse, and 6400 PS was used to provide illumination. The optical quantum flux density (*PFD*) was set at 1000 μmol/(m^2·s) (human light source). The temperature of leaf chamber was controlled at (25±1) ℃, the concentration of CO_2 was (380±10) μmol /mol, and the relative humidity (*RH*) was 50%. The formulas (3.1, 3.2):

$$L_s = 1\text{-}C_i / C_a \qquad 3.1,$$

where C_a is the concentration of CO_2 in air;

$$WUE = P_n/T_r \qquad 3.2,$$

were used to calculate stomatal limit (L_s) and water utilization ratio (*WUE*), respectively.

As can be seen from Table 3.4, under different concentrations of NaCl, P_n, T_r, and *WUE* of the two kinds of pumpkins all decreased, and the decreasing amplitude increased with the increase of NaCl concentration. Under 60 and 120 mmol/L NaCl, the P_n fraction and T_r of Yanzhen pumpkin were 13.95% and 46.41% lower than that under the exposure, and 12.16% and 37.30% lower than that under the exposure, respectively. P_n and T_r of Miben pumpkin decreased by 29.92% and 64.61%, respectively, and 25.98% and 46.56%, respectively, compared with the control.

As can be seen from Table 3.4, G_s, L_s and C_i of the two kinds of pumpkin significantly decreased under different concentrations of NaCl. Under 60 and 120 mmol/L NaCl, G_s and L_s of Yanzhen pumpkin decreased by 10.30% and 41.97% and 3.20% and 15.50%, respectively, compared with the control.

Compared with the control, G_s of Miben pumpkin decreased 23.19% and 67.35% respectively, and L_s decreased 9.82% and 31.13% respectively. The results showed that the decreasing range of G_s and Ls in pumpkin increased with the increase of salt stress concentration, and the decreasing range of Miben pumpkin was larger than Yanzhen pumpkin. Although the Ls decreased under salt stress, the influence of stomatal factors decreased, but the CO_2 into plant cells decreased with the G_s decreased.

After 7 days of salt stress with different concentrations, the changes of chlorophyll content of the two kinds of pumpkin were different (Fig. 3.7). The contents of chlorophyll a, b and chlorophyll in the leaves of Yanzhen pumpkin increased with the increase of NaCl concentration.

Table 3.4. Effects of different concentrations on photosynthetic characteristics and water use of pumpkins[1]

Variety	NaCl (mmol/L)	*Pn* [μmol/(m^2·s), CO_2]	*Tr* [mmol/(m^2·s), H_2O]	*WUE* (mol/mol, CO_2/H_2O)	*Gs* [mol/(m^2·s), H_2O]	C_i (μmol/mol, CO_2)	L_s
Yanzhen	0 (CK)	16.35a	4.30a	3.80a	0.1110a	226.16e	0.405a
	60	14.07b	3.78b	3.72ab	0.0996b	238.76d	0.372b
	120	8.76d	2.70d	3.25c	0.0644d	256.70b	0.324d
Miben	0 (CK)	16.45a	4.38a	3.83a	0.1121a	226.26e	0.485a
	60	11.46c	3.18c	3.60b	0.0853c	248.41	0.346c
	120	5.79e	2.30e	2.52d	0.0362e	279.50a	0.264e

Note: Different letters after the same column of numbers indicate significant difference at the 0.05 level

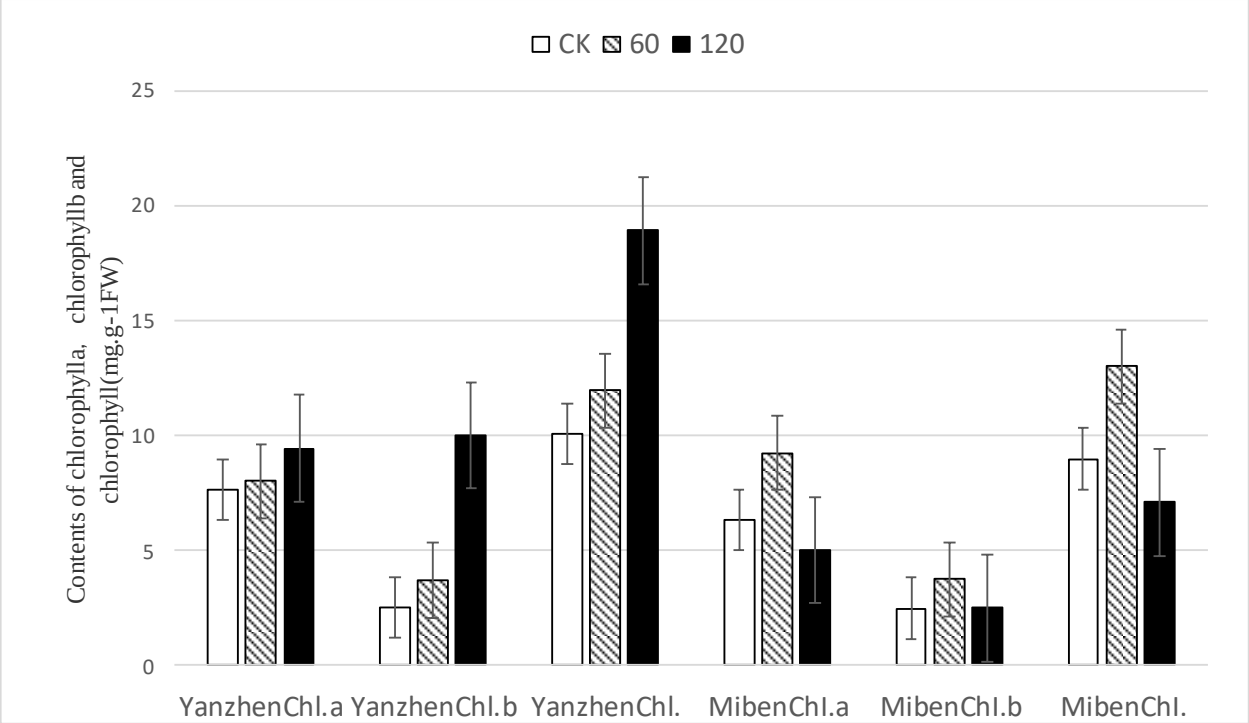


Fig. 3.3. Effects of NaCl stress on contents of chlorophyll a, chlorophyll b and chlorphyll in leaves of Yanzhen pumpkin and Miben pumpkin

Although chlorophyll a decreased under the condition of 120 mmol/L NaC1, it was still significantly higher than the control. The contents of chlorophyll a, b and chlorophyll in the leaves of Miben pumpkin showed the same trend of change, that is, they all increased significantly under the condition of 60 mmol/L NaCl, while the contents of the three decreased with the increase of NaCl concentration. Under the condition of 120 mmol/L NaCl, the contents of chlorophyll a and chlorophyll were significantly lower than the control. Chlorophyll b content was reduced to an undesirable level compared with the control.

3.2.2. Effects of salt stress on plasma membrane permeability and MDA content

The production rate of O_2^- is accelerated after salt stress, which leads to membrane system damage and cell damage, and the content of MDA in the body is increased. As shown in Figure 3.4, MDA content in the leaves of the two kinds of pumpkin increased with the increase of salt treatment concentration, indicating that the plasma

membrane peroxidation level was aggravated by salt treatment at different concentrations.

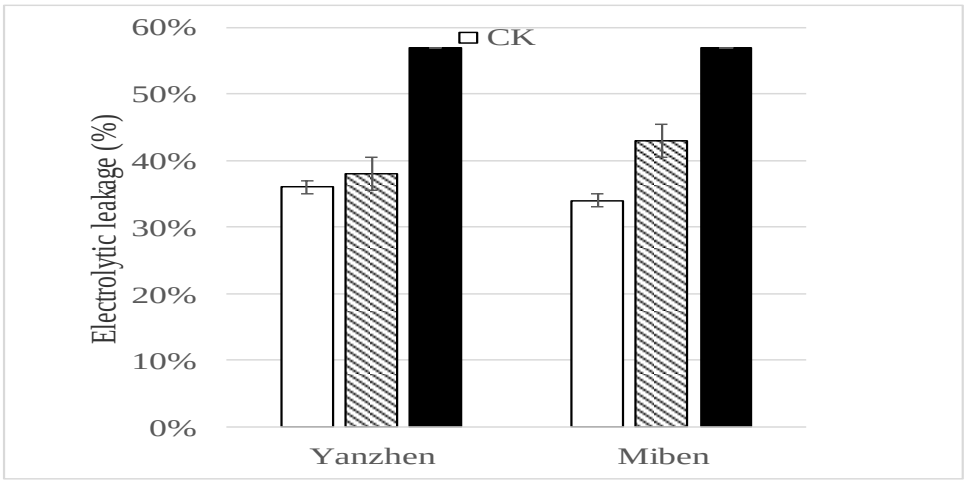


Fig.3.4. Effects of NaCl stress on electrolytic leakage of Yanzhen pumpkin and

Miben pumpkin MDA content in the leaves of Miben pumpkin treated with different concentrations of NaCl was slightly higher than that of Yanzhen pumpkin. The relative permeability of plasma membrane also increased with the increase of salt concentration (Fig. 3.5).

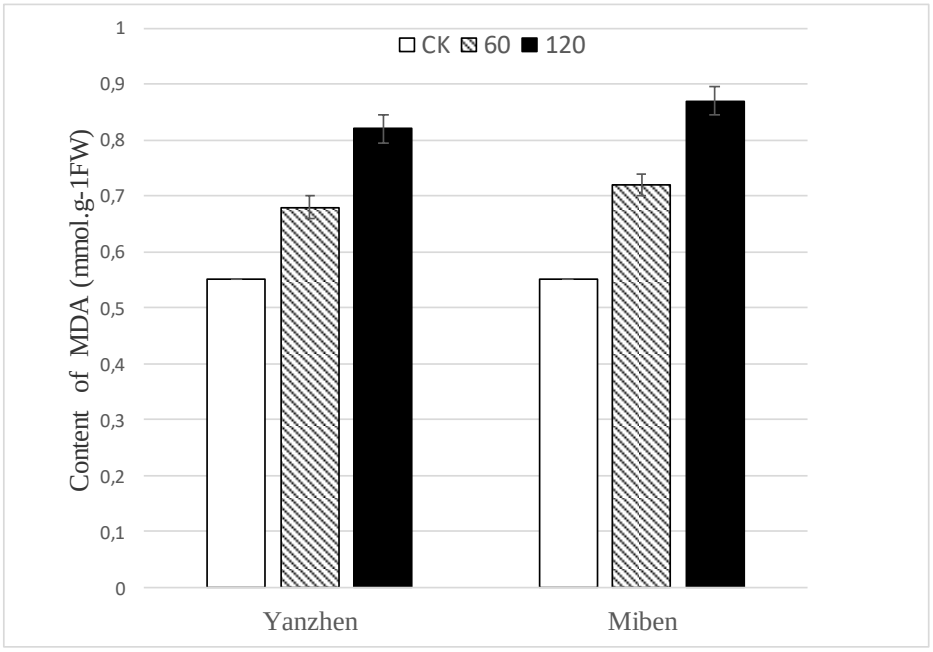


Fig. 3.5. Effects of NaCl stress on MDA content in Yanzhen pumpkin and Miben pumpkin

After 7 days of treatment, the plasma membrane permeability of Yanzhen pumpkin leaves was 106% and 159% of that of the control. The damage effect of NaCl on the plasma membrane of Miben pumpkin was 130% and 171%, which indicated that the damage effect of NaCl on the plasma membrane of Miben pumpkin was greater than that of Yanzhen pumpkin.

The above changes in plasma membrane permeability and MDA content of Yanzhen and Miben pumpkin leaves after NaCl stress indicate that Yanzhen pumpkin has strong salt tolerance. In the later stage of stress, the production and scavenging of reactive oxygen species and free radicals have reached a balance under the action of antioxidant enzymes and antioxidants. The production rate of O_2^- and the content of MDA remained unchanged at a certain level, while the salt tolerance of Miben pumpkin was weak, the production and removal of reactive oxygen species and free radicals could not reach balance, the lipid peroxidation of root membrane was intensified, and the content of malondialdehyde was high (He Songtao et al., 2020).

3.2.3. Effects of salt stress on contents of free proline and soluble sugar in pumpkin

Soluble sugar and proline are important osmotic substances that regulate osmotic potential and water potential of plants under salt stress. After 7 days of NaCl stress, the content of proline in leaves of two pumpkin seedlings was significantly higher than that of the control. Under the conditions of 60 mmol/L NaCl and 120 mmol/L NaC1, the proline content in Yanzhen pumpkin leaves was 4.3 times and 6.2 times that of the control, respectively. However, the proline content of Miben pumpkin showed no obvious increase trend 4, and its content was 4.3 times and 4.6 times of that of the control (Figure 3.6).

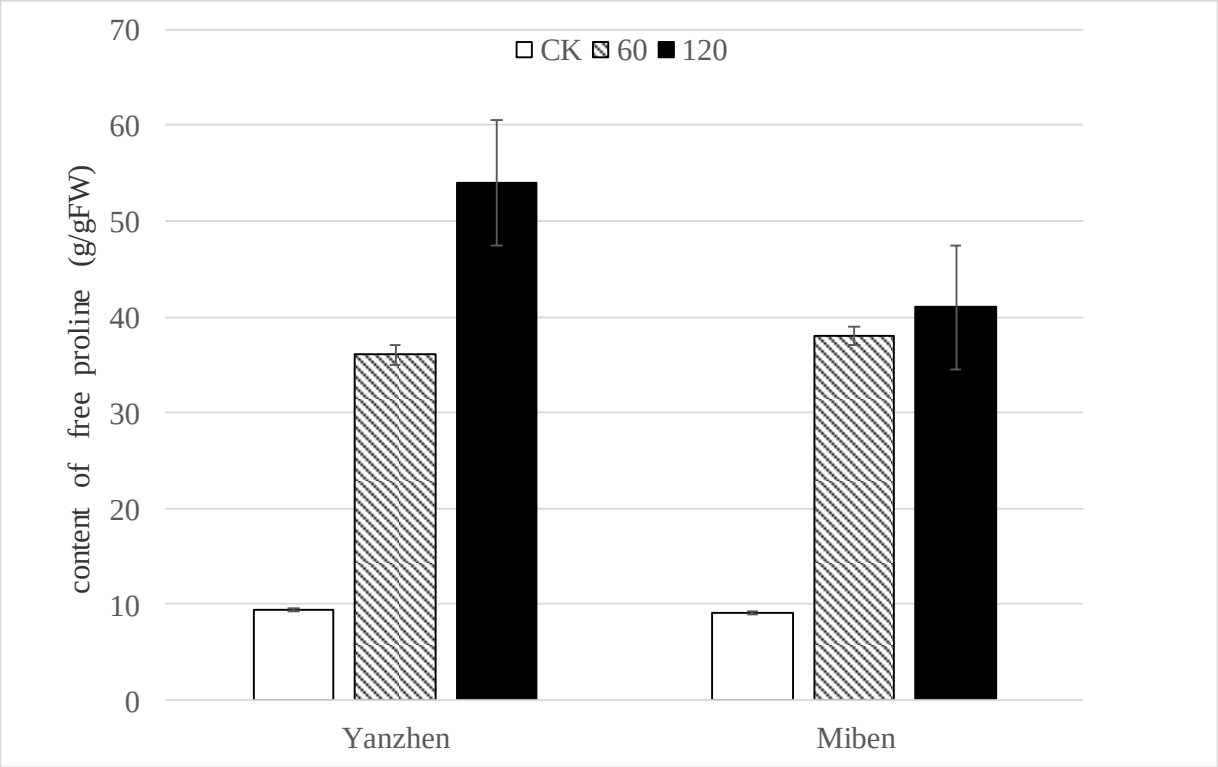


Fig. 3.6. Effects of NaCl stress on free proline content in Yanzhen pumpkin and Miben pumpkin

Figure 3.7 shows that with the increase of NaCl concentration, the soluble sugar content in the Yanzhen pumpkin leaves gradually increases. At 120 mmol/L NaCl, the content of soluble sugar in 'salt anvil' pumpkin leaves was 2.5 times that of the control. The soluble sugar content of Miben pumpkin only increased significantly under the condition of 60 mmol/L NaCl, while the soluble sugar content of Miben pumpkin leaves under the condition of high concentration of 120 mmol/L NaCl had little difference with the control.

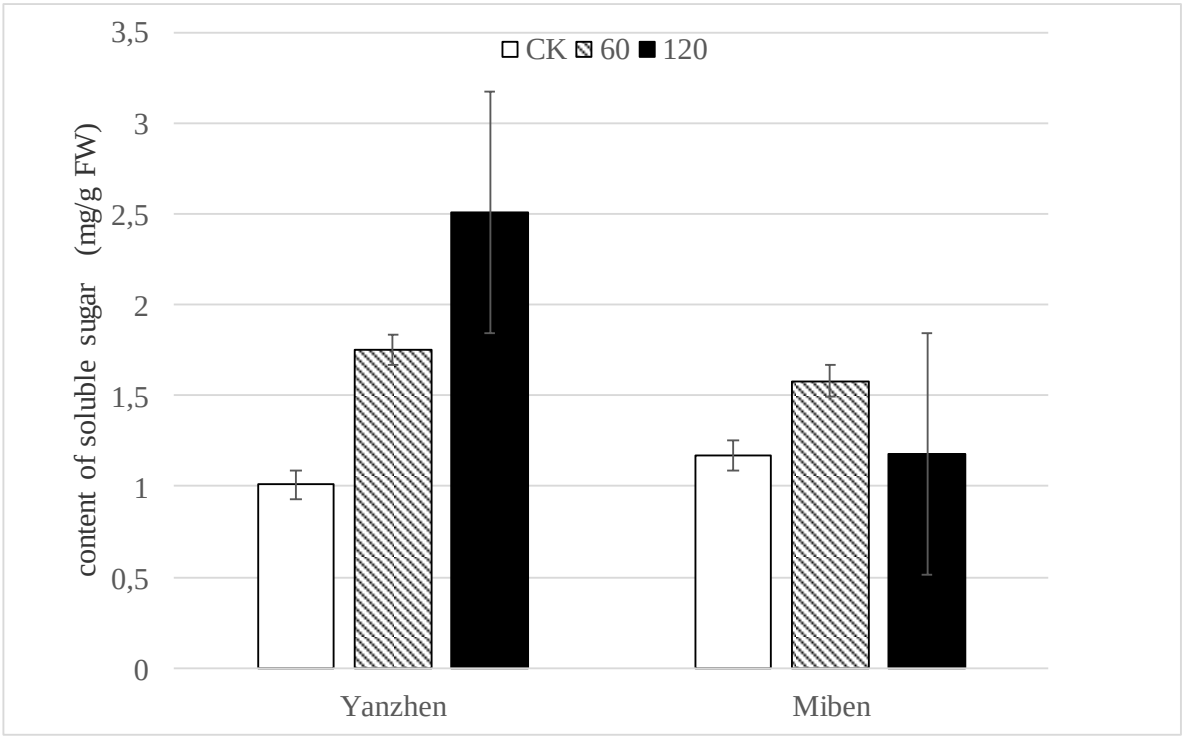


Fig.3.7. Effects of NaCl stress on content of soluble sugar in Yanzhen pumpkin and Miben pumpkin

Under normal conditions, there was little difference in the contents of proline and soluble sugar between the two pumpkins, but under salt stress, especially under high concentration of salt stress, the accumulation of proline and soluble sugar in the leaves of Yanzhen pumpkin was significantly higher than that of Miben pumpkin. It can be seen that the ability of these two genotypes of pumpkin seedlings to accumulate organic solutes is significantly different when NaCl stress exceeds a certain concentration.

These results indicate that after NaCl stress, more soluble sugar and proline can be synthesized in the root system of Yanzhen to maintain osmotic balance under salt stress, with strong osmotic regulation ability. However, the contents of soluble sugar and proline of Miben pumpkin were increased by the internal reaction at the beginning of salt stress. With the increase of salt stress concentration, the root system suffered more damage. However, more soluble sugars and prolines could not be synthesized to maintain osmotic balance.

3.2.4. Generalization of the results of the study of the effect of salt stress on the exchange of organic compounds and the flow of leading physiological processes in pumpkin

Therefore, plant salt tolerance is controlled by multiple genes, and the mechanism of salt tolerance is different in different plants. Salt harms plants mainly through ion toxicity, osmotic stress and nutrient deficiency. The salt damage index can be used as a suitable index to identify the salt tolerance of perennial plants. Leaf salt damage index was often used as an indicator of salt tolerance. In this study, it was concluded that the salt damage index of pumpkin under salt stress, as a morphological index of salt tolerance, was simple and easy to detect the strength of salt tolerance of pumpkin directly and transformed the traditional apparent salt tolerance evaluation into a standardized evaluation method with quantitative indicators, which increased the accuracy and reliability of salt tolerance evaluation.

Salt stress has a significant effect on the whole growth and development cycle of plants, among which the seedling stage is an important period of plant growth and development, which is more sensitive to environmental changes. In this study, two different concentrations of NaCl were used to treat different pumpkin varieties. Combined with the growth conditions of the whole growth cycle and the degree of salt damage, the concentration of salt stress resistance identification of pumpkin seedlings was analysed. The results showed that the salt tolerance of Yanzhen showed in each stage of growth.

Traditional studies on salt stress mainly focus on the effects on plant leaves. This study explored the correlation between salt damage grade and dry weight by studying the relationship between salt damage grade and the whole growth cycle of pumpkin, indicating that the degree of salt damage can be reflected through the changes of stem diameter, leaf size, dry weight and other traits, which can provide a certain reference for the research on the mechanism of salt tolerance and the breeding of salt-tolerant varieties of pumpkin.

The relative growth of plants under stress conditions can reflect the strength of stress resistance. The larger the relative growth, the stronger the resistance to adversity. In this study, through the salt tolerance test of different pumpkin varieties

under the same cultivation conditions, the comprehensive analysis of the salt resistance of different pumpkin varieties is concluded that there are obvious differences in the salt resistance of different pumpkin varieties. The salt resistance of different pumpkin varieties is analysed from the salt damage index and salt damage rate index Yanzhen strong salt resistance. Through the comparison of this experiment, the salt tolerance of Yanzhen is better than Miben, indicating that its salt tolerance has a greater use value, it is necessary to make further research on its stock characteristics, to use it as a salt tolerant melon stock variety.

Salt stress reduces photosynthesis in plants by affecting the diffusion of CO_2 to binding sites, changing the structure and function of organelles responsible for photoreaction, changing the chemical process of dark reaction and inhibiting the transfer of assimilative products. The results of this experiment showed that salt stress could lead to the decrease of G_s and Tr in pumpkin leaves, which was conducive to reducing water loss in plants and enhancing their resistance to salt stress. The decrease of G_s will cause the transport of CO_2 required for photosynthesis to mesophyll cells to be blocked. However, the reduction of plant Pn caused by salt stress also reduces the CO_2 required for photosynthesis, resulting in the accumulation of CO_2 in plant cells and the increase of C_i value. The decrease of L_s indicated that stomatal restriction was not the main factor affecting plant photosynthesis under salt stress. The decreased photosynthesis of tested materials under salt stress may lead to the reduction of carbohydrate and inhibit plant growth.

Cytoplasmic membrane permeability controls the flow of substances into and out of cells. The normal exchange of substances in living cells mainly depends on the normal maintenance of cytoplasmic membrane permeability. Under salt stress, the plasma membrane of plant cells is the first to be damaged, resulting in the destruction of the selective permeability of the membrane, that is, the permeability becomes larger, and many organelle functions are destroyed. It is generally believed that there are two main reasons for the damage to plasma membrane under salt stress. First, the membrane lipid peroxidation caused by salt stress destroys the plasma membrane structure. As an indicator of lipid peroxidation level, MDA has been accepted by people. The stronger the lipid peroxidation, the higher the MDA content and the

greater the membrane permeability. Therefore, it is generally believed that the amount of MDA accumulation and the increase of membrane permeability are negatively correlated with the salt tolerance of varieties, that is, the more MDA accumulation, the greater the increase of membrane permeability and the weaker the salt tolerance, because the increase of surface MDA accumulation and membrane permeability can be used to compare the surface address between varieties. In addition, MDA affects cell ion absorption and accumulation by affecting membrane permeability and membrane transport proteins. MDA content and membrane permeability of two kinds of pumpkin under NaCl stress both increased with the increase of salt concentration, indicating that NaCl stress enhanced membrane peroxidation of plant leaves, damaged membrane system and increased membrane permeability. Under the same concentration of NaCl stress, the increase of MDA content and membrane permeability was different between Miben pumpkin and Yanzhen pumpkin. Another major reason why NaCl damages the plasma membrane structure is the excessive accumulation of Na^+ in cells, which replaces the Ca^{2+} that can stabilize and protect the plasma membrane. Although NaCl has little effect on the total content of Ca^{2+} in the test, it may affect the morphology of Ca^{2+} in cells and thus affect the structure of the plasma membrane.

The growth of plants and the yield of crops are fundamentally determined by the intensity of photosynthesis, and the absorption of light energy, the formation of electron transport, the formation of reducing capacity and the formation of primary photosynthates are all related to the structure of chloroplasts. Chloroplast is the most sensitive organelle of cells to salt and is vulnerable to salt stress. Chlorophyll content of plant leaves under salt stress is not only directly related to the photocontracting process of plants, but also one of the important physiological indexes to measure the salt tolerance of plants. Increase of chlorophyll content in leaves is to reduce the physiological disorder caused by salt stress and slow down the physiological interference caused by the relaxation of the binding between protein and chlorophyll, which is another adaptive physiological effect of salt-tolerant plants in saline environment. Synthesis of chlorophyll required proline, and the large amount of proline accumulated in cells under salt stress was conducive to chlorophyll synthesis.

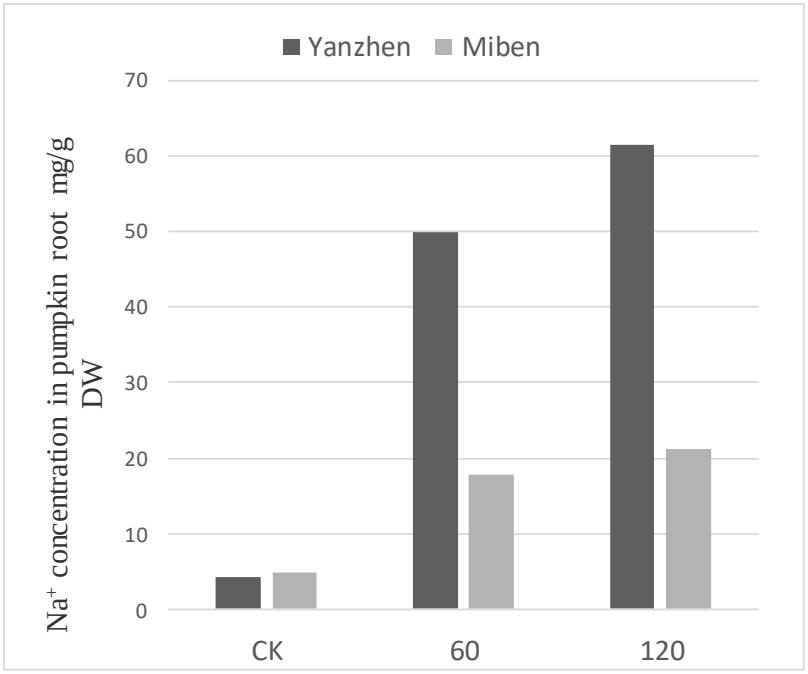
Stressed soybean seedlings with NaCl, the contents of chlorophyll a (chla), chlorophyll b (chlb), total chlorophyll (chlT) and carotenoid (Car) increased, and the chla/chlb values decreased to varying degrees, which was more obvious under severe stress. Chlorophyll content increased with the increase of NaCl concentration. However, when the concentration is greater than 20 $g \cdot L^{-1}$, it decreases again. Salt stress can reduce plant chlorophyll content. Chlorophyll a was the most sensitive to salinity, followed by chlorophyll b, and carotenoid was the least sensitive. Reduction of chlorophyll was mainly caused by the degradation of chlorophyll b by chlorophyll enzymes, while the content of chlorophyll a and carotenoid was less affected. The results of this experiment showed that the contents of chlorophyll b and chlorophyll in the Yanzhen pumpkin leaves showed the same change trend, that is, with the increase of NaCl concentration, the content of chlorophyll a also increased under the condition of low concentration of NaCl (60 mmol/L) compared with the control, but decreased when the NaCl concentration was 120 mmol/L. The contents of chlorophyll a, chlorophyll b and chlorophyll in the leaves of Miben pumpkin showed the same change trend, that is, they all increased significantly under the condition of low concentration of NaCl (60 mmol/L), and the contents of the three decreased with the increase of NaCl concentration. On the one hand, these differences indicated that the variation in degree of leaf chlorophyll content was related to plant species, salt species and concentration, on the other hand, salt stress could affect leaf chlorophyll content, and the initial response of plants to salt stress was polymorphic. Therefore, chlorophyll content can only be used as a reference index of salt tolerance.

3.3. Studies on ion absorption and accumulation characteristics of pumpkin plants under NaCl stress

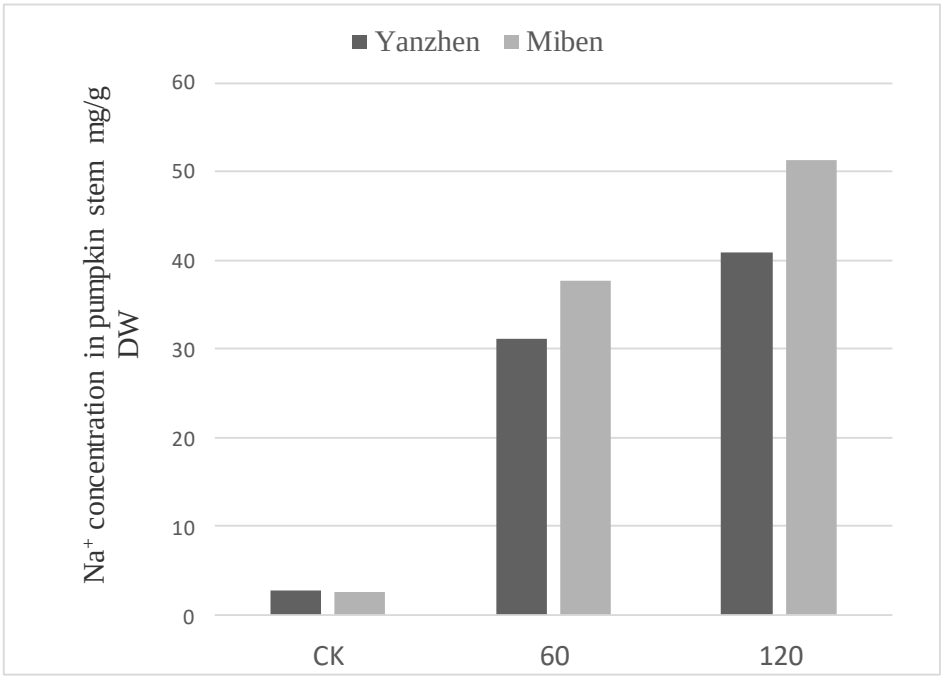
3.3.1. Accumulation of Na^+ in different organs of pumpkin under NaCl stress

As can be seen from Figure 3.8, the Na^+ content in organs of the two varieties was basically the same without NaCl stress, both at relatively low levels. After 60 mmol/L NaCl stress and 120 mmol/L NaCl stress, the Na^+ content in all organs of the two pumpkin plants increased significantly, but the increase amplitude was different.

A


Yanzhen
Miben
Na+ concentration in pumpkin root mg/g DW
70
60
50
40
30
20
10
0
CK
60
120

B


Yanzhen
Miben
Na+ concentration in pumpkin stem mg/g DW
60
50
40
30
20
10
0
CK
60
120

C

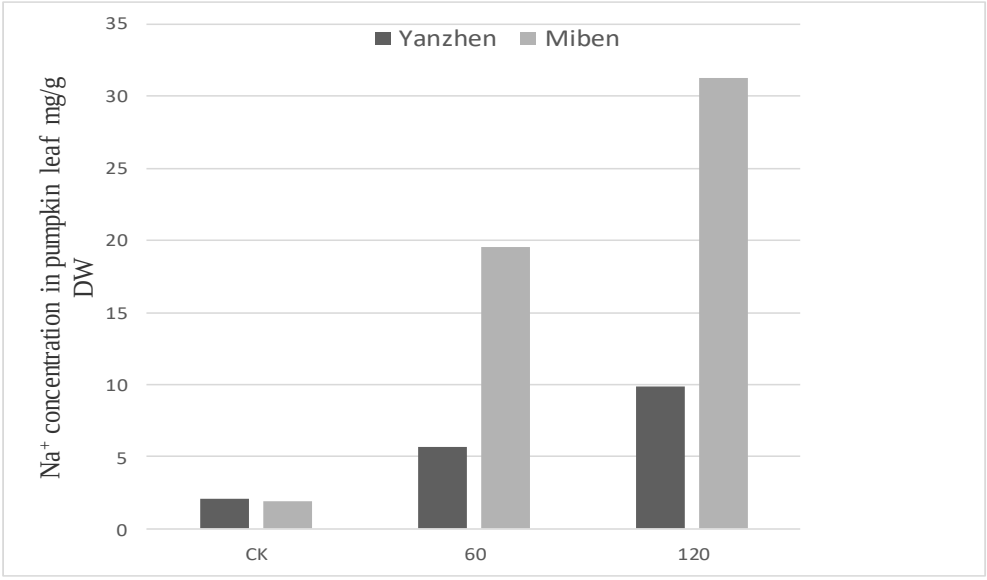


Fig. 3.8. Effects of NaCl stress on Na^+ content in roots, stems and leaves of Yanzhen pumpkin and Miben pumpkin

The content of Na^+ in Miben pumpkin leaves increased to 5.2 times and 8.3 times of the control, respectively, while that in Yanzhen pumpkin was 1.5 times and 2.8 times of the control. The content of Na in the stem of Miben pumpkin was 10.4 times and 14.1 times of that in the control, while that in Yanzhen pumpkin was 8.1 times and 10.6 times of that in the control. The content of Na^+ in the root of Miben pumpkin was 3.7 times and 4.4 times of that of the control, and that of Yanzhen pumpkin was 11.7 times and 14.3 times of that of the control. In conclusion, under high concentration (120 mmol/L) NaCl stress, the Na^+ content in the leaves of Miben pumpkin reached 31.24 mg/gDW, while the Na^+ content in the leaves of Yanzhen pumpkin was only 9.86 mg/gDW, which was only one third of that in Miben.

The Miben pumpkin material accumulated most of the Na in the above-ground part, while the Yanzhen pumpkin material accumulated more Na in the underground part. In other words, Miben accumulates more than 80% Na^+ in the aboveground part, while Yanzhen accumulates more than 50% Na^+ in the underground part. Significantly higher than black-seeded squash. The order of Na^+ content in the organs of Yanzhen

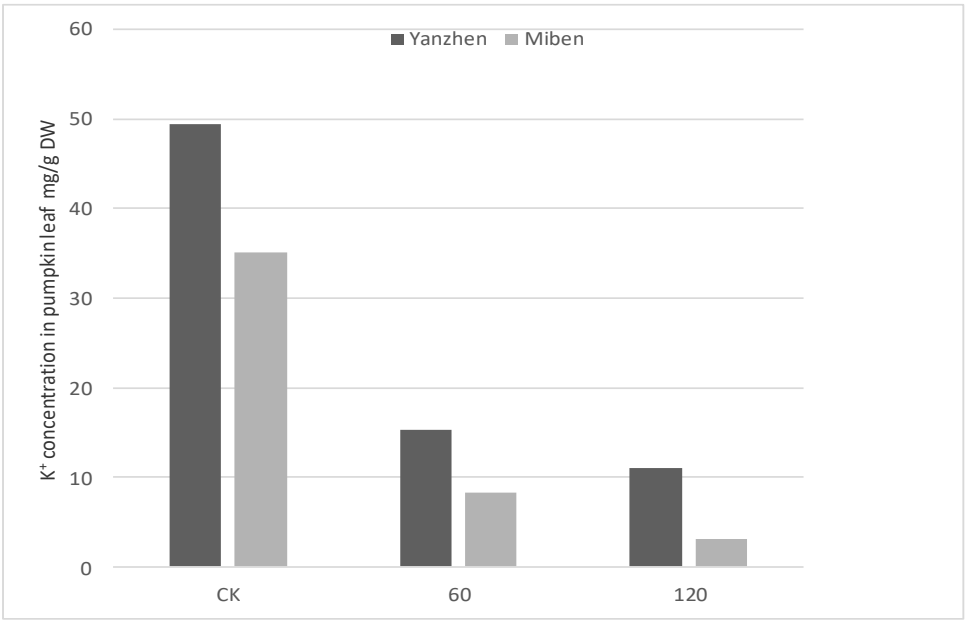
plants from high to low was root → stem → leaf, while the order of Na^+ content in the organs of Yanzhen plants from high to low was stem → leaf → root.

The above results indicate that the organs of Yanzhen and Miben pumpkin that accumulate Na^+ after being subjected to NaCl stress are different. Yanzhen plants mainly accumulate Na^+ in roots, while Miben plants mainly accumulate Na^+ in leaves.

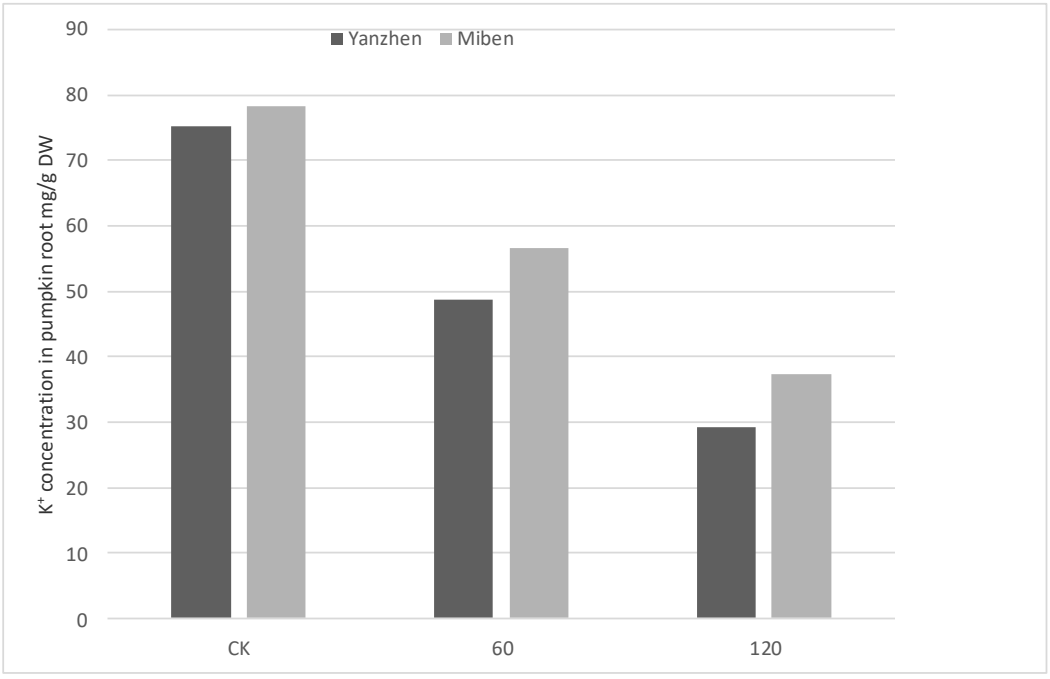
3.3.2. Accumulation of K^+ in different organs of pumpkin under NaCl stress

Compared with the control, the content of K^+ in each organ of the two pumpkins under NaCl stress decreased, and the greater the NaCl concentration, the greater the decrease in K^+ content, because the accumulation of Na^+ inhibited the absorption of K^+ after NaCl stress. The distribution of K^+ content in each part of two pumpkin plants was stem → root→ leaves. After salt treatment, the content of K^+ in the two pumpkin varieties was significantly lower than that in the control (Fig. 3.9).

A



B



C

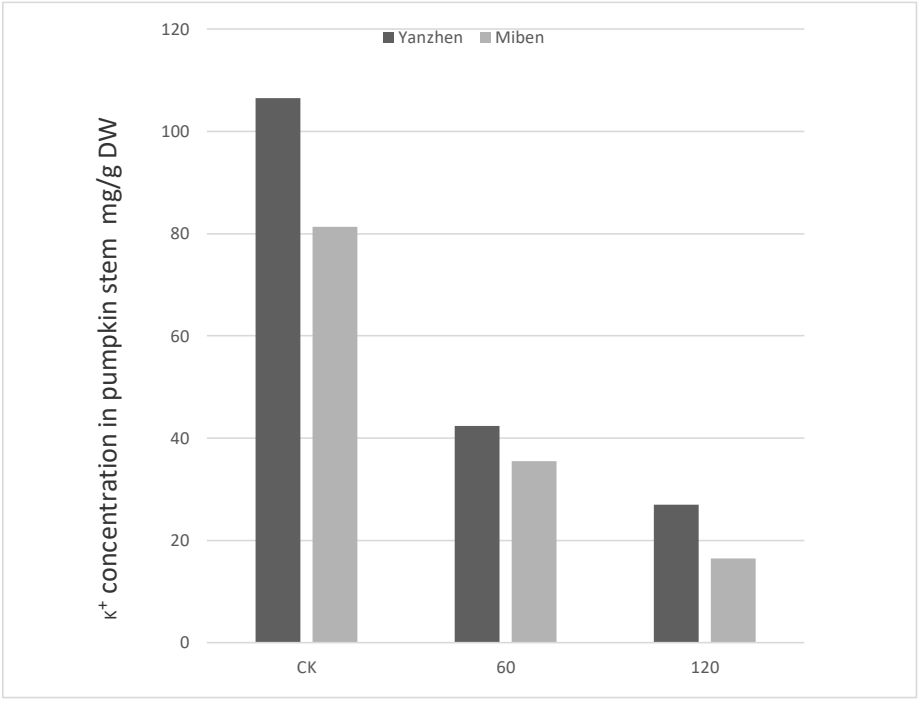


Fig. 3.9. Effects of NaCl stress on K^+ content in roots, stems and leaves of Yanzhen pumpkin and Miben pumpkin

The content of K^+ in the root of all pumpkin varieties decreased less, and the content of K^+ in the leaves decreased most obviously. Studies have shown that maintaining higher K^+ in the aboveground can help reduce the damage of Na^+ to plants. K^+/Na^+ ratio can better reflect this effect. K^+/Na^+ was the highest in stems, the second in leaves, and the lowest in roots of all pumpkin varieties (Tabl. 3.5).

Table 3.5. Effects of Nacl stress on theK^+/Na^+ of pumpkin[1]

Varieties	K^+/Na^+		
	Root	Stem	Leaf
0 mmol/L NaCl (CK)			
Miben	16.17ab	30.92c	18.68b
Yanzhen	17.58a	37.35c	23.38c
60 mmol/L NaCl			
Miben	3.16cd	0.941a	0.42a
Yanzhen	0.98bc	1.36c	2.68ab
120 mmol/L NaCl			
Miben	1.76a	0.32b	0.10c
Yanzhen	0.47a	0.66b	1.12c

Note: Different letters indicate significant differences at P < 0.05 by Duncan's multiple range test

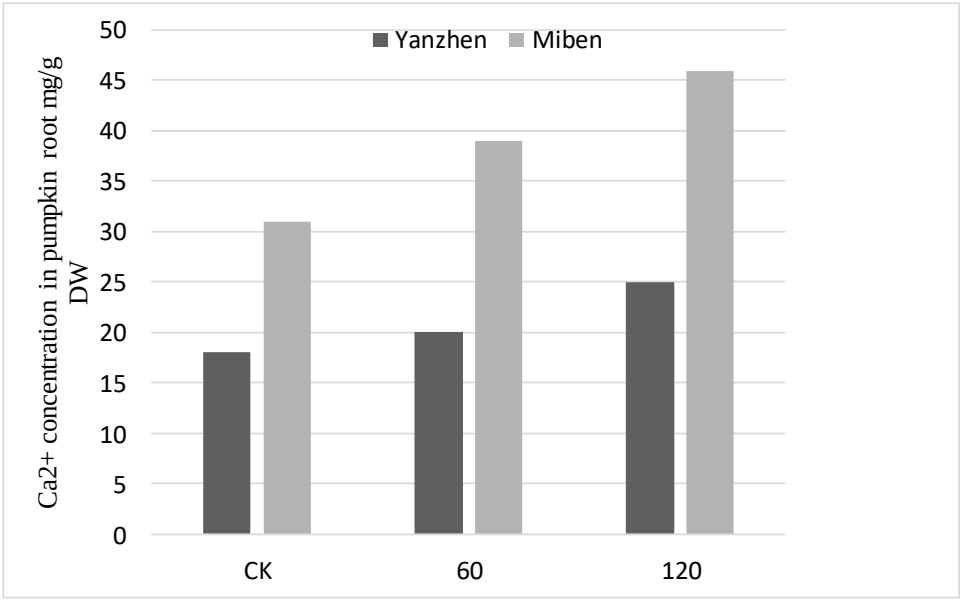
After NaCl treatment, K^+/Na^+ of all pumpkin varieties decreased significantly. The K^+/Na^+ in the root of the Miben pumpkin material with fewer Na^+ accumulation in the underground part is significantly higher than that of the Yanzhen pumpkin with more Na^+ accumulation in the underground part. There was no significant difference in K^+/Na^+ in stem of all pumpkin varieties. However, the K^+/Na^+ content of the Yanzhen pumpkin was higher, which was mainly due to the lower Na^+ content in the leaves.

After salt stress, the content of K^+ in the ground and in the lower part showed a decreasing trend compared with the control. The magnitude of the decline varied among different varieties. The decrease of K^+ content in underground part was significantly higher than that in aboveground part. The decrease of K^+ content in underground part was 39%-67%, while that in aboveground part was 2%-25%, indicating that K^+ ions were mainly transported to aboveground part after absorption, which was beneficial to avoid aboveground part K^+ nutrient deficit and ensure the normal physiological activities of plants.

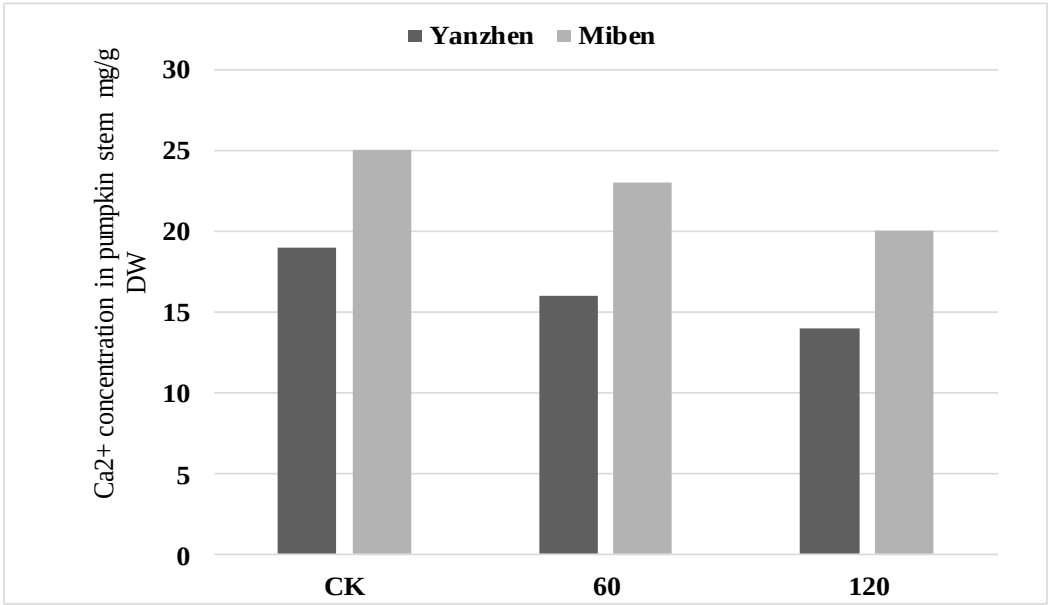
3.3.3. Ca^{2+} accumulation in different organs of pumpkin under NaCl stress

As can be seen from Figure 3.10, the leaves of the two varieties are the main sites of Ca^{2+} accumulation. After NaCl stress, the order of Ca^{2+} content in the organs of Yanzhen plants was leaf → root → stem, while the order of Ca^{2+} content in the organs of Miben plants was root → leaf → stem, with great changes in the roots and increased content, while the changes in other organs were not obvious.

A



B



C

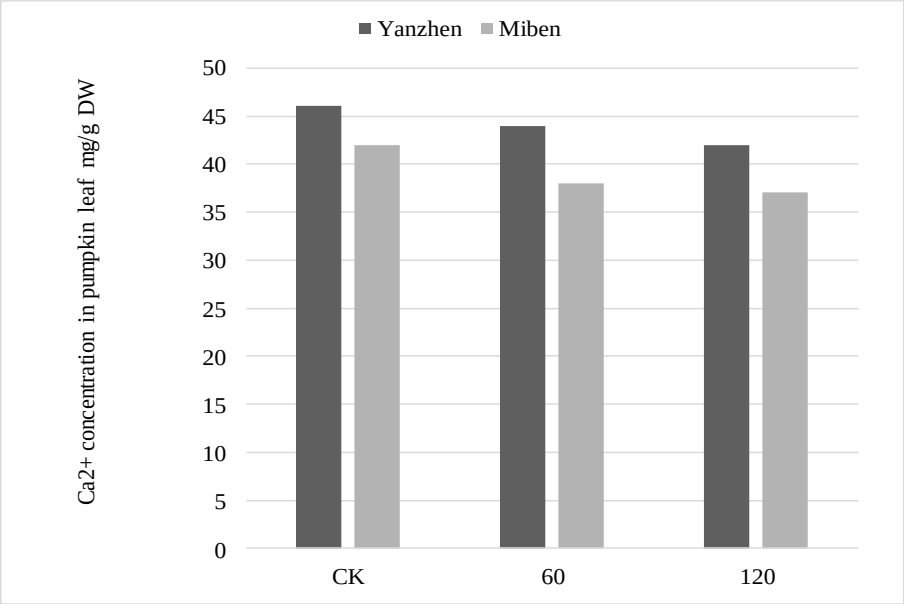


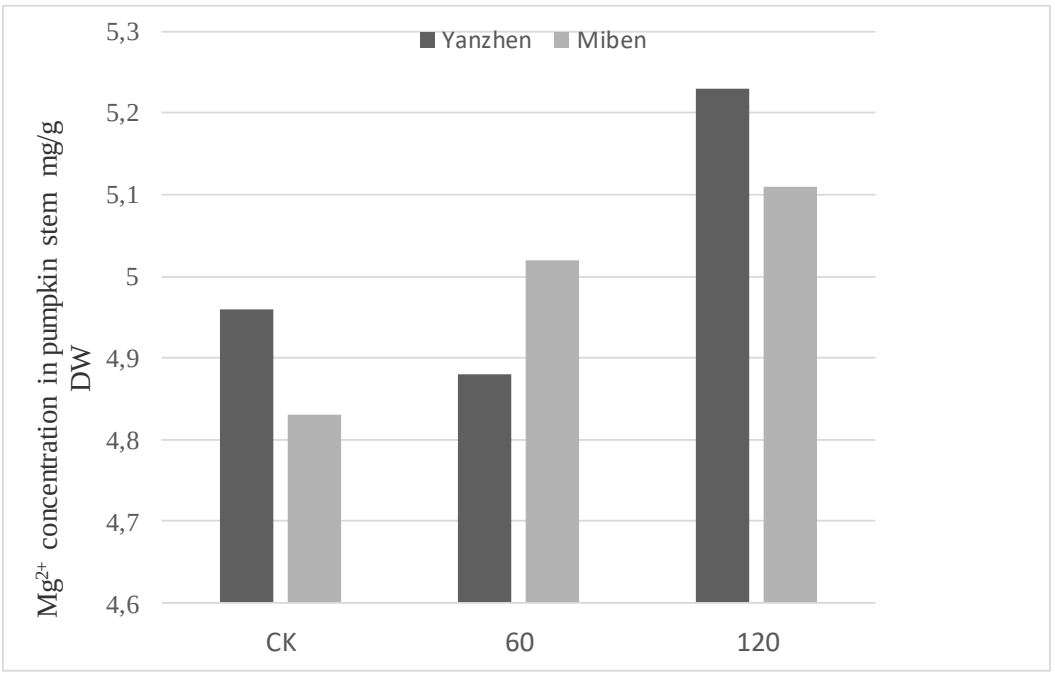
Fig. 3.10. Effects of NaCl stress on Ca^{2+} content in roots, stems and leaves of Yanzhen pumpkin and Miben pumpkin

Ca^{2+} is a stabilizer of biofilms and plays an important role in maintaining the integrity and selectivity of biofilms under salt stress. The content of Ca^{2+} in pumpkin of different varieties increased slightly or remained unchanged. In both control and salt treatment, Ca^{2+} content in above-ground parts was higher than that in underground parts, indicating that different varieties could selectively absorb beneficial nutrients under salt stress and avoid nutrient deficiency in above-ground parts. There was no significant difference in Ca^{2+} content in the same part of pumpkin between different varieties.

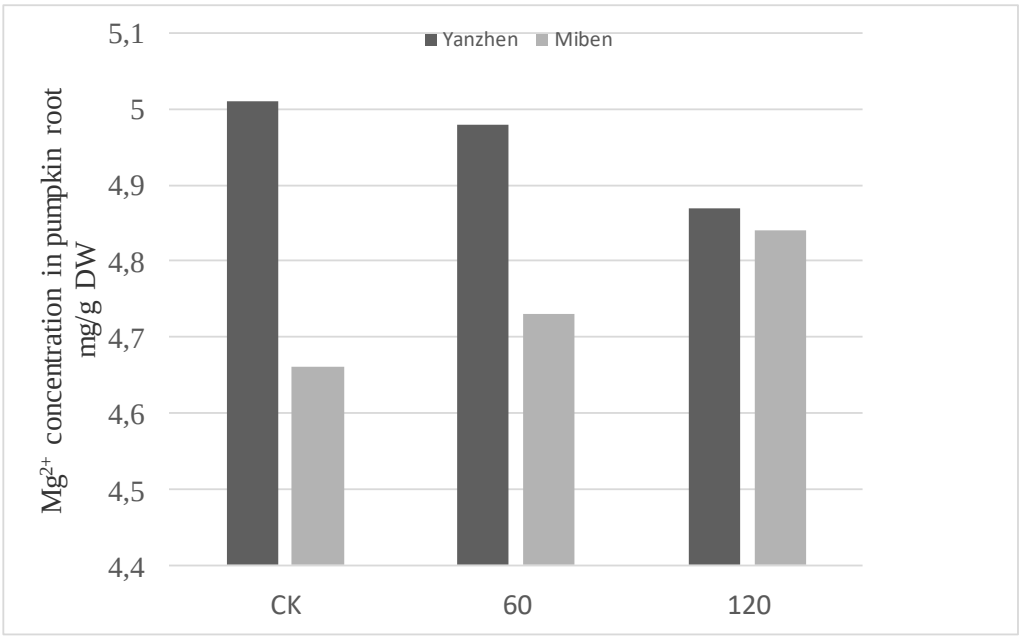
3.3.4. Accumulation of Mg^{2+} in different organs of pumpkin under NaCl stress

As can be seen from Figure 3.11, Mg^{2+} ions of the two varieties mainly accumulate in the leaves. After NaCl stress, Mg^{2+} content in the roots, stems and leaves of the two kinds of pumpkin had little change, while Mg^{2+} content in the leaves of Yanzhen plants increased slightly. Mg^{2+} content in Miben leaves decreased significantly.

A


Yanzhen
Miben
5,3
5,2
5,1
5
4,9
4,8
4,7
4,6
Mg2+ concentration in pumpkin stem mg/g DW
CK
60
120

B


Yanzhen
Miben
5,1
5
4,9
4,8
4,7
4,6
4,5
4,4
Mg2+ concentration in pumpkin root mg/g DW
CK
60
120

C

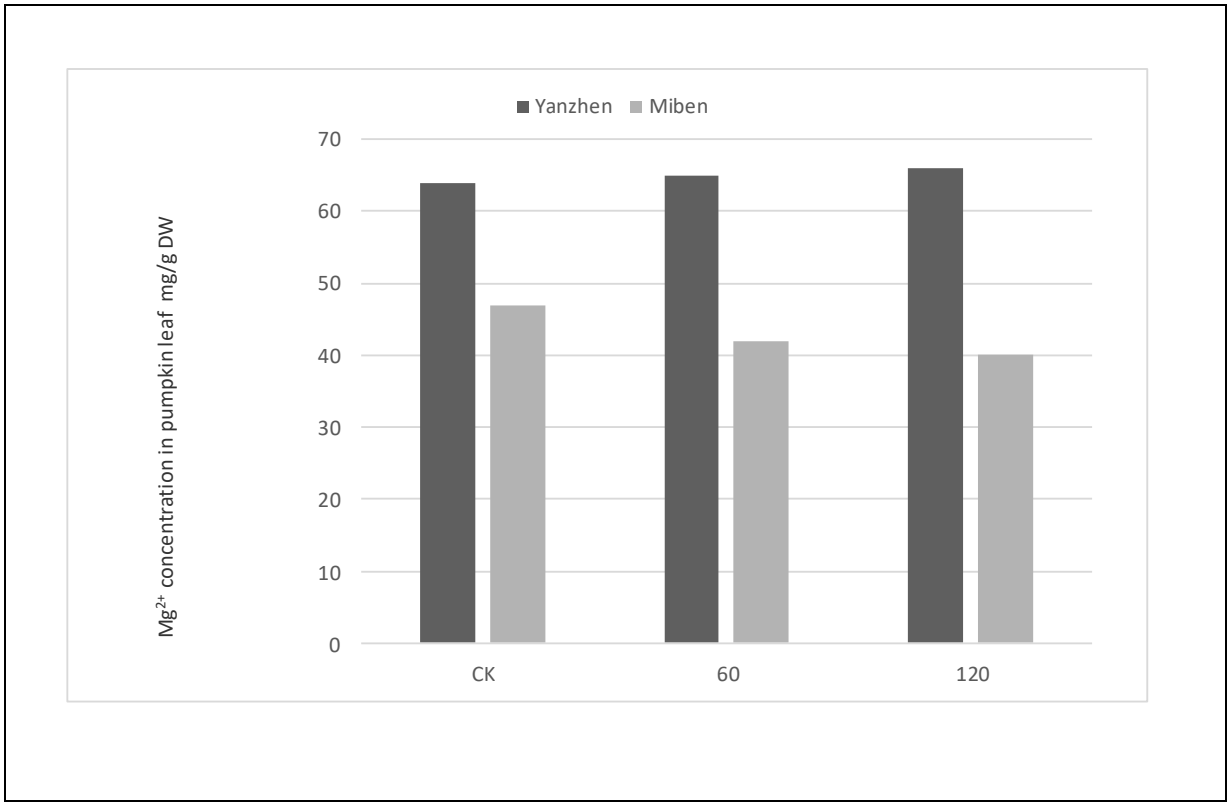


Fig. 3.10. Effects of NaCl stress on Mg^{2+} content in roots, stems and leaves of Yanzhen pumpkin and Miben pumpkin

3.3.5. Accumulation of ions in aboveground and underground parts of pumpkin after NaCl stress

The K^+/Na^+ in aboveground and underground parts of two kinds of pumpkin decreased with the increase of NaCl concentration, and the decrease in aboveground part was much greater than that in underground part. Compared with the control, the above-ground K^+/Na^+ decreased by 97% and the subsurface K^+/Na^+ decreased by 92% when treated with 120 mmol/L NaCl. The above ground K^+/Na^+ decreased by 98% and below ground by 91%. Under control and NaCl treatment, the ratio of Na^+ to other ions in aboveground and underground parts of Yanzhen pumpkin was much lower than that of Miben pumpkin (Table 3.6), indicating that the overall Na^+ expulsion ability of Yanzhen pumpkin was higher than that of Miben pumpkin.

Table 3.6. K^+/Na^+ and Na/cations of shoot and root, and shoot/ root of cations in Miben and Yanzhen after NaCl stress

Variety	NaCl (mmol/L)	K^+/Na^+		Na/cations		Shoot/root ratio			
		shoot	root	shoot	root	Na	K	Ca	Mg
Yanzhen	0	33.27	3.54	0.01	0.16	0.34	4.10	9.13	3.73
	60	6.78	0.63	0.07	0.48	0.44	4.79	10.11	3.75
	120	1.11	0.29	0.28	0.61	1.27	4.78	7.50	3.01
Miben	0	28.00	2.67	0.02	0.20	0.37	3.84	9.57	2.81
	60	5.64	0.35	0.09	0.62	0.33	5.32	9.80	3.11
	120	0.56	0.23	0.45	0.63	2.07	4.96	6.85	2.02

The content ratio (S/R) of various ions in aboveground and underground parts reflects the transport of ions from roots to aboveground parts. Compared with the control, the (S/R) ratios of Na^+ and K^+ of both kinds of pumpkin were increased under NaCl treatment, but the (S/R) ratio of K^+ content was decreased under 120 mmol/L NaCl condition compared with the low concentration of NaCl condition. The (S/R) ratio of Ca^{2+} content in the two kinds of pumpkin was higher than that in the control at 60 mmol/L NaCl, but lower than that in the control at 120 mmol/L NaCl. The (S/R) ratio of Mg^{2+} content in Yanzhen pumpkin was higher than that in the control under 60 mmol/L NaCl condition and lower than that in the control under 120 mmol/L NaCl condition. The (S/R) ratio of Mg^{2+} content in Miben pumpkin was higher than that in the control at 60 mmol/L NaCl and lower than that in the control at 120 mmol/L NaCl. This indicated that low concentration (60 mmol/L) of NaCl increased the transport of various ions to the aboveground part. With the increase of NaCl concentration, the equilibrium of ion transport and distribution was broken, and numerous Na^+ ions entered and transported and had antagonistic effects on the transport of other cations.

3.3.6 Changes of Na^+/K^+, Na^+/Ca^{2+} and Na^+/Mg^{2+} ratios in different parts of pumpkin after NaCl stress

After salt treatment, the results of comparison of Na^+/K^+, Na^+/Ca^{2+} and Na^+/Mg^{2+} ratios in the roots, stems and leaves of the two pumpkins showed that the absorption capacity of Miben pumpkin to nutrient elements under salt stress was lower than that of Yanzhen pumpkin (Table 3.7). With the increase of salt concentration, the ratio of Na^+ to the three nutrient elements in the two kinds of pumpkin was increased. The increased ratio of Na^+ to nutrient elements came from two aspects: one is the net increase of Na^+ in the tissue, the other is the decrease of nutrient element level. Compared with the control, the ratio of root, stem and leaf of Miben pumpkin increased significantly under 120 mmol/L NaCl conditions than that of 'salt stock' pumpkin. Under 120 mmol/L NaCl, the Na^+/K^+, Na^+/Ca^{2+} and Na^+/Mg^{2+} ratios in all organs of Miben pumpkin were significantly higher than those of Yanzhen pumpkin.
Salt ions absorbed by plant roots are transported up the ground by transpiration. Water is dissipated through transpiration, while salt ions remain in the leaves. Saline soil will accumulate a large amount of Na^+ in the plant. Since non-halophyte plants do not have the salt-discharge structure unique to halophyte plants such as salt glands, the excessive accumulation of salt in the leaves will eventually lead to the appearance of salt damage symptoms. Excessive accumulation of Na^+ in non-halophytic plants is harmful, which can affect the balance of other ions in the plant and inhibit plant growth.

The salt tolerance of non-halophones is related to the ability to prevent the absorption of salt ions and control the transport of salt ions to the over ground part. The basic strategy is to preferentially accumulate salt ions in the lower part of roots and stems and mature leaves to prevent excessive accumulation in upper leaves and ensure high K^+ content in young tissues. K^+, Ca^{2+} and Mg^{2+} are beneficial elements in plant body, and their balance is very important for the normal growth and development of plant. Therefore, the salt tolerance of non-halophyte plants is mainly determined by limiting the uptake of Na by plant roots and maintaining a low level of Na^+ in leaves.

Table 3.7. Effects of stress on Na^+/K^+, Na^+/Ca^{2+} and Na^+/Mg^{2+} of Yanzhen and Miben[1]

Variety	NaCl (mmol/L)	Root			Stem			Leaf		
		Na^+/K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}	Na^+/K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}	Na^+/K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}
Yanzhen	0	0.28f	1.88d	1.23e	0.04g	0.05f	0.13f	0.02f	0.03e	0.09e
	60	1.85e	4.61c	3.69d	0.20f	0.45e	0.79e	0.05ef	0.04e	0.12e
	120	2.91d	6.21b	6.07bc	1.16c	1.49c	2.73c	0.60c	0.64c	1.53c
Miben	0	0.37f	1.64d	1.18e	0.03g	0.08f	0.16f	0.03ef	0.04e	0.11e
	60	3.50c	8.13a	6.02bc	0.27e	0.68d	1.21d	0.06e	0.06e	0.18e
	120	6.37a	8.74a	9.07a	2.24a	3.25a	6.24a	1.25a	1.17a	3.40a

Note: Different letters indicate significant differences at $P < 0.05$ by Duncan's multiple range test

The ionic radii and hydration energy of Na^+ and K^+ are similar, showing obvious antagonistic effect. The accumulation of Na^+ in non-halozoan plants under salt stress is often accompanied by the decrease of K^+ content, showing the characteristics of selective absorption. As for the phenomenon of high K^+/Na^+ value in crops with high salt tolerance, it may be related to the dynamic enzymes in the root vesicle membrane and plasma membrane.

The reason may be the increase of ATPase activity in the root membrane at the tip, and the transmembrane gradient of protons formed promoted K^+ to enter the cytoplasm through the K^+ channel. Moreover, the Na^+/H^+ exchange gate on the plasma membrane is activated, which accelerates the absorption of K^+ and the emission of Na^+, and improves the selectivity of K^+. The selective absorption of ions and the ability to regulate regionalized distribution of plants were closely related to their salt tolerance, and there were significant differences among different varieties.

From the distribution of ions in different organs of the two kinds of pumpkin, it can be seen that the absorption of Na^+ by the root system of Yanzhen pumpkin is slightly lower than that of Miben pumpkin, while the transport of Na^+ to the ground part, especially the leaves, of Yanzhen pumpkin is significantly lower than that of Miben pumpkin. Na has a strong competitive effect on the absorption of K^+, resulting in the reduction of K^+ content under NaCl stress. Most salt-tolerant plants showed selective absorption of K^+ and less absorption of Na^+, and had a strong ability to transport K^+ to the overground part. In addition, it has also been reported that salt treatment does not affect or increase K^+ content in leaves, and this opposite experimental result may be caused by different experimental conditions. After 7 days of NaCl stress, the absorption and transport of K^+ to the overground part of both Yanzhen pumpkin and Miben pumpkin were inhibited. Under the conditions of 60 and 120 mmol/L NaC1, Na^+/K^+ in root and leaf of Yanzhen pumpkin was significantly lower than that of Miben pumpkin, indicating that Yanzhen pumpkin had a higher selective ability to absorb and transport K^+.

Ca^{2+} plays an important role in plant salt tolerance, which helps to improve the K^+ selective absorption capacity of plants and maintain the K^+/Na^+ ratio. NaCl in nutrient solution can lead to Ca^{2+} deficit in plants. Salt treatment had no significant

effect on Ca^{2+} content in olive leaves, while Ca^{2+} content in roots increased. It has also been reported that with the increase of salt concentration in soil, the content of Ca^{2+} in the leaves of salt-sensitive plants decreased, while the content of Ca^{2+} in salt-tolerant plants increased or remained unchanged. In this experiment, with the increase of NaCl concentration, Ca^{2+} content in two kinds of pumpkin organs decreased first in stem, then in leaf, while there was no significant decrease in root, which indicated that within the range of NaCl concentration, Ca^{2+} content in pumpkin roots was absorbed by NaCl. However, Ca^{2+} transport to the aboveground part was gradually inhibited with the increase of NaCl concentration. In addition, Ca^{2+} plays an important role in maintaining the integrity and selectivity of biofilms. The decreased level of intracellular Ca^{2+} in plants suggests that the level of intracellular Ca^{2+} in the plasma membrane is decreased. It may be replaced by Na^{+}, which will reduce the selective permeability of the membrane and directly lead to numerous salt ions entering. Compared with Miben pumpkin, Yanzhen pumpkin maintains higher Ca^{2+} level under salt stress and non-salt stress, which may be closely related to its strong salt tolerance, and plays an important role in maintaining low Na^{+}/K^{+} ratio in the body.

It has been reported that salt treatment can cause Mg^{2+} deficiency, but the competitive effects of Na^{+} and Mg on absorption and transportation have not been reported. Compared with the control, there was little difference in Mg^{2+} content in the organs of Yanzhen pumpkin, indicating that NaCl had little effect on Mg absorption and retransport in salt anvil pumpkin. Under the conditions of 60 and 120 mmol/L NaC1, Mg^{2+} content in the root of Miben pumpkin increased significantly, while decreased significantly in the leaves, which may be caused by high concentration of NaCl affecting Mg^{2+} transport in the root of Miben pumpkin to the leaves to some extent.

In a saline environment, a high concentration of NaCl usually changes the nutrient balance of plants, leading to changes in the ratio of salt ions to nutrient elements, such as the increase of the ratio of Na^{+}/K^{+}, Na^{+}/Ca^{2+}, Na^{+}/Mg^{2+} and Cl^{-}/NO^{3-}, affecting the normal physiological metabolism of cells. The results of this experiment showed that, compared with the control, the absorption of various nutrient elements in the root system of the two kinds of pumpkin decreased, and the content of various

nutrient elements in the above-ground parts also decreased correspondingly, which was exactly reflected in the changes of the ratios of Na^+/K^+, Na^+/Ca^{2+} and Na/Mg in different organs.

The reasons may be the following: 1. The competition between salt ions and nutrient elements reduces the absorption of nutrient elements. 2. Salt ions affect the ion selectivity of biofilm and interfere with the absorption of nutrient elements by roots. The comparison of these three ratios between the two kinds of pumpkin showed that the Miben pumpkin had lower absorption capacity of nutrient elements under salt stress than the «salt anvil» pumpkin. Under salt stress conditions, Yanzhen pumpkin can maintain a relatively good nutritional status, which may be one of the important reasons for its strong salt resistance.

In summary, the main reason for the difference in salt tolerance between the two kinds of pumpkin plants is the difference in Na^+ content in various organs under NaCl stress. The Yanzhen mainly accumulates in the root system, while the Miben mainly accumulates in the stem, resulting in different changes in the absorption and accumulation of K^+, Ca^{2+} and Mg^{2+} in various organs, and the reduction range of K^+/Na^+ is also different. Thus, the growth inhibition degree of Yanzhen is smaller than that of Miben.

3.4. Genetic aspects of adaptation and response of pumpkins to salt stress

3.4.1. Identification and expression analysis of WRKY gene family in pumpkin under salt stress

Whole genome identification and physicochemical properties' analysis of WRKY family members of pumpkin

Firstly, bioinformatics website Pfam was used to search WRKY DNA-binding domain (PF003106), which was unique to WRKY transcription factors. Then, we downloaded the seed sequence of PF003106 from Pfam and the whole pumpkin genome data published by Boyce Thompson Institute (BTI) and China National Vegetable Engineering and Technology Research Center (Beijing) in 2017. Finally, we used the hidden Markov model to compare the sequences between the two. The comparison method is to set e-value&lt on HMMER software. le-5, screened the

protein sequences containing PF03106 (WRKY DNA-binding domain) in the whole pumpkin genome. After screening, 86 candidate WRKY transcription factors were obtained.

The methods of naming transcription factors in current studies are different. One is the homologous Arabidopsis transcription factor name as the method of naming transcription factors in other species, and the other is the method of naming transcription factors based on the location of genes on chromosomes. The different naming methods lead to different descriptions of the same transcription factor in different literatures. In this study, Arabidopsis thaliana was named by comparison according to its position on chromosomes (Table 3.8).

Table 3.8. The basic information of WRKY gene family in pumpkin

Gene ID	Gene name	Number of amino acid	Molecular weight/Da	Theoretical pI	GRAVY	Category
CmoCh11G018380.1	CmWRKY1_2	282	31.6034	6.36	-0.989	IIc
CmoCh02G007110.1	CmWRKY1_1	302	34.2082	8.72	-0.846	IIc
CmoCh02G005990.1	CmWRKY10_2	704	78.9721	10.05	-0.805	IIc
CmoCh20G011350.1	CmWRKY10_1	277	31.2418	5.12	-0.778	IIc
CmoCh11G003510.1	CmWRKY11	309	35.5204	6.43	-0.526	IId
CmoCh03G010490.1	CmWRKY12_1	342	37.7083	6.36	-0.716	IIc
CmoCh14G002380.1	CmWRKY12_2	645	69.7790	6.56	-0.638	IIc
CmoCh06G009630.1	CmWRKY12_3	212	24.1542	8.37	-0.557	IIc
CmoCh02G005040.1	CmWRKY15	253	27.6142	9.88	-0.923	IId
CmoCh19G011100.1	CmWRKY16_1	306	34.8666	5.50	-0.789	IIe
CmoCh11G020440.1	CmWRKY16_2	490	53.7599	6.51	-0.423	IIe
CmoCh03G004310.1	CmWRKY16_3	196	22.3505	6.06	-0.400	IIe
CmoCh01G017100.1	CmWRKY18_1	419	45.9452	9.06	-1.168	IIa
CmoCh13G000170.1	CmWRKY18_2	272	30.7892	5.75	-0.650	IIa
CmoCh01G007370.1	CmWRKY18_3	321	35.3758	8.55	-0.434	IIa
CmoCh01G018760.1	CmWRKY2_1	271	30.7571	10.09	-1.125	I
CmoCh09G002470.1	CmWRKY2_2	206	24.0012	9.01	-0.136	I
CmoCh16G009820.1	CmWRKY20_1	278	31.6311	6.11	-0.685	I
CmoCh06G006650.1	CmWRKY20_2	590	62.8662	6.04	-0.770	I
CmoCh08G003110.1	CmWRKY21_1	323	35.3261	9.65	-0.610	IId
CmoCh19G000310.1	CmWRKY21_2	288	32.4316	5.15	-0.867	IId
CmoCh02G018140.1	CmWRKY21_3	317	35.0726	6.74	-0.612	IId
CmoCh04G007420.1	CmWRKY21_4	488	53.9967	6.53	-0.567	IId
CmoCh16G006040.1	CmWRKY21_5	325	35.8200	8.31	-0.901	IId
CmoCh03G010450.1	CmWRKY21_6	523	58.0568	5.86	-0.872	IId
CmoCh14G021260.1	CmWRKY22_1	273	30.8332	5.62	-3847	IIe
CmoCh06G011130.1	CmWRKY22_2	350	37.9779	9.69	-0.661	IIe

Cont. Table 3.8

Gene ID	Gene name	Number of amino acid	Molecular weight/Da	Theoretical pI	GRAVY	Category
CmoCh14G009620.1	CmWRKY23	183	20.7818	7.04	-0.826	IIc
CmoCh06G007190.1	CmWRKY24	257	28.1278	10.19	-0.826	IIc
CmoCh03G009820.1	CmWRKY26	418	46.0315	7.68	-0.536	IIc
CmoCh15G011240.1	CmWRKY27_1	312	34.5088	8.76	-0.736	IIe
CmoCh18G013290.1	CmWRKY27_2	162	17.9500	5.45	-0.628	IIe
CmoCh16G000740.1	CmWRKY27_3	169	18.7630	5.01	-0.537	IIe
CmoCh04G017660.1	CmWRKY3_1	337	38.1620	6.31	-0.943	I
CmoCh05G003550.1	CmWRKY3_2	298	33.4513	5.90	-0.769	I
CmoCh18G004080.1	CmWRKY30	273	27.1245	7.60	-0.453	I
CmoCh17G003670.1	CmWRKY31_1	250	22.5544	6.22	-0.402	IIb
CmoCh14G016120.1	CmWRKY31_2	147	16.6155	9.13	-0.620	IIb
CmoCh06G015650.1	CmWRKY31_3	271	29.3418	9.82	-0.837	IIb
CmoCh10G005000.1	CmWRKY32_1	539	59.8265	6.93	-0.642	I
CmoCh11G004590.1	CmWRKY32_2	245	28.0273	6.37	-0.557	I
CmoCh08G012540.1	CmWRKY32_3	308	34.8923	5.62	-0.921	I
CmoCh14G000290.1	CmWRKY33	420	46.2380	6.65	-0.842	I
CmoCh04G007680.1	CmWRKY35_1	506	54.9409	6.38	-0.842	IIe
CmoCh16G006300.1	CmWRKY35_2	325	36.5674	5.30	-0.803	IIe
CmoCh07G000480.1	CmWRKY35_3	83	9.7804	9.84	-0.771	IIe
CmoCh03G014640.1	CmWRKY35_4	305	33.0305	9.65	-0.522	IIe
CmoCh05G004350.1	CmWRKY38	446	48.7375	7.03	-0.714	III
CmoCh11G009610.1	CmWRKY4_1	284	31.8611	8.42	-0.635	III
CmoCh18G011450.1	CmWRKY4_2	342	38.2854	5.99	-0.554	III
CmoCh10G008630.1	CmWRKY4_3	356	39.6938	5.77	-0.921	III
CmoCh14G001690.1	CmWRKY4_4	59	6.7900	6.00	-0.787	III
CmoCh01G001920.1	CmWRKY40_1	540	56.1731	8.30	-0.426	I
CmoCh12G003170.1	CmWRKY40_2	589	64.0627	6.13	-0.402	I
CmoCh09G005200.1	CmWRKY41	169	18.8830	9.37	-0.131	III
CmoCh03G005730.1	CmWRKY42_1	263	28.3229	9.84	-0.681	IIb
CmoCh07G003110.1	CmWRKY42_2	470	51.3855	6.24	-0.774	IIb
CmoCh14G010700.1	CmWRKY5	543	59.5090	8.18	-0.617	IIc
CmoCh17G011970.1	CmWRKY51	275	31.0896	9.19	-0.862	IIc
CmoCh04G000970.1	CmWRKY55	223	25.8565	8.18	-0.614	III
CmoCh04G029190.1	CmWRKY58_1	220	24.9208	9.07	-0.563	I
CmoCh15G002580.1	CmWRKY58_2	491	55.1012	7.29	-0.908	I
CmoCh19G009380.1	CmWRKY6_2	375	41.4286	6.85	-0.876	IIb
CmoCh11G018890.1	CmWRKY6_3	262	30.4714	5.95	-0.822	IIb
CmoCh06G010490.1	CmWRKY6_4	679	70.2107	5.92	-0.843	IIb
CmoCh17G005420.1	CmWRKY6_5	201	20.7622	9.71	-0.711	IIb
CmoCh14G021630.1	CmWRKY6_6	518	57.6006	8.81	-0.552	IIb
CmoCh08G001250.1	CmWRKY6_1	189	20.9633	8.59	-0.784	IIb
CmoCh16G010370.1	CmWRKY62	333	37.9279	6.13	-0.645	IIb
CmoCh19G010450.1	CmWRKY65_1	317	35.1788	5.03	-0.574	IIe

Cont. Table 3.8

Gene ID	Gene name	Number of amino acid	Molecular weight/Da	Theoretical pI	GRAVY	Category
CmoCh11G019840.1	CmWRKY65_2	309	34.5105	5.30	-0.951	IIe
CmoCh05G004640.1	CmWRKY67	384	42.9390	9.36	-0.747	III
CmoCh01G002870.1	CmWRKY68_1	303	33.8433	5.29	-0.416	IIc
CmoCh13G009630.1	CmWRKY68_2	211	24.4003	5.44	-0.482	IIc
CmoCh10G003940.1	CmWRKY7	307	29.8878	6.21	-0.191	III
CmoCh06G001750.1	CmWRKY70_1	316	36.4226	8.60	-0.641	III
CmoCh04G011250.1	CmWRKY70_2	357	39.7931	9.69	-0.734	III
CmoCh18G005080.1	CmWRKY72	322	36.1107	6.32	-0.647	IIb
CmoCh14G014930.1	CmWRKY75_1	503	54.8600	8.12	-0.862	IIc
CmoCh14G007400.1	CmWRKY75_2	278	31.6311	6.11	-0.654	IIc
CmoCh13G006140.1	CmWRKY8	367	41.2245	6.99	-0.583	IIc
CmoCh16G003930.1	CmWRKY81	80	8.8167	9.46	-0.948	IIc
CmoCh01G016200.1	CmWRKY84	256	29.3682	5.13	-0.876	IIc
CmoCh10G001980.1	CmWRKY9_1	429	44.4134	8.59	-0.788	IIb
CmoCh04G005460.1	CmWRKY9_2	317	36.0275	8.28	-0.826	IIb
CmoCh20G009780.1	CmWRKY97	940	108.0014	5.92	-0.970	IIb

Bioinformatics analysis showed that the isoelectric points of the 86 CmWRKY proteins ranged from 5.01 (CmWRKY27-3) to 10.19 (CmWRKY24), and the protein size ranged from 59 to 940 (CmWRKY97) amino acids, with an average number of amino acids of The molecular weight (MW) varied from 6.7900 to 108.0014 kD.

Construction of pumpkin WRKY transcription factor phylogenetic tree

Phylogenetic studies of WRKY transcription factors in many model plants have been completed, among which the WRKY transcription factor family in Arabidopsis Thaliana is the most extensively studied. Arabidopsis WRKY transcription factor family was divided into 3 groups according to the composition of conserved domain, and the second group was further divided into 5 groups. The purpose of classification is to study the evolutionary relationship and function of WRKY transcription factors in plants. In this study, 1 to 2 Arabidopsis WRKYs and pre-screened pumpkin WRKYs were randomly selected from each group of all Arabidopsis taxa. MEGAX software was used to construct phylogenetic trees of WRKY transcription factor families of all pumpkin and part of Arabidopsis thaliana, as shown in Figure 3.11. According to the classification method of WRKY transcription factors in Arabidopsis thaliana, WRKY

transcription factors from the same branch of pumpkin were assigned to the same group in pumpkin. Finally, all 86 pumpkin WRKY transcription factors were divided into three groups.

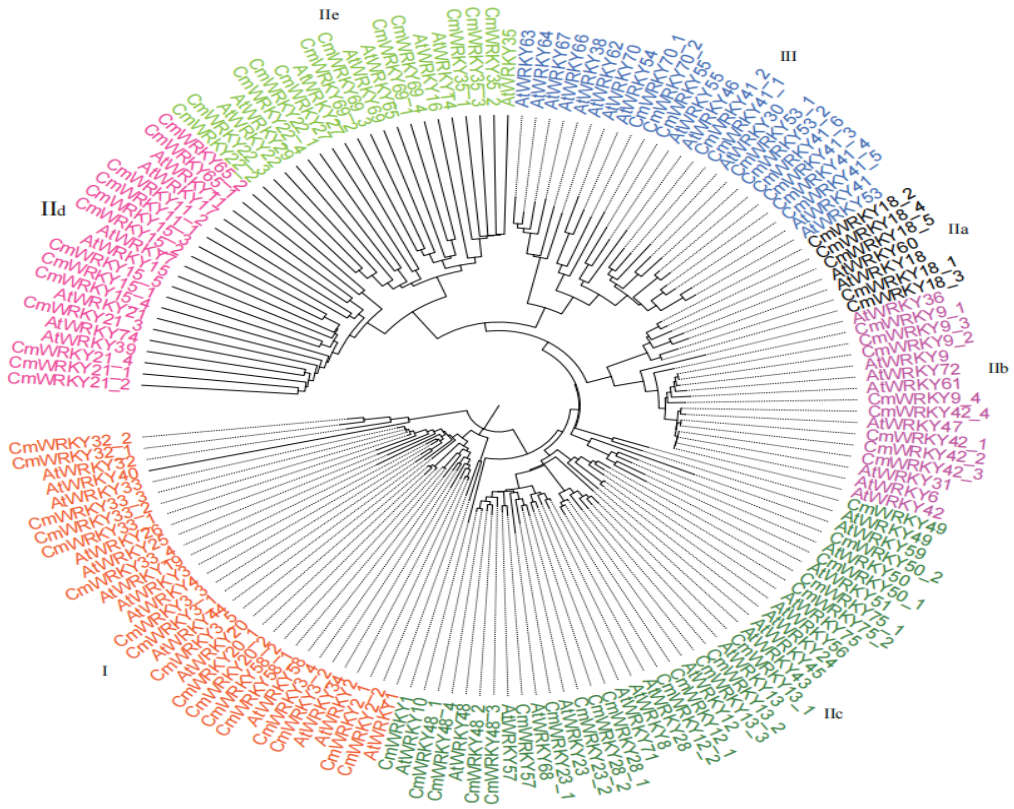


Fig. 3.11. Phylogenetic tree of WRKY family members in pumpkin and *Arabidopsis thaliana*

Class I WRKY has high homology with AtWRKY2. Studies have shown that AtWRKY2 plays a role in osmotic stress responses induced by NaCl and mannitol, so it is speculated that WRKY in class I pumpkin has a similar role. Class II WRKY transcription factors can be divided into five subclasses according to the zinc finger domain. It can be seen from the evolutionary tree that WRKys of class IIa and AtWRKY18/60 have higher homology. In Arabidopsis thaliana, AtWRKY18 and AtWRKY40 bind directly to the transcription-like kinase CRK5 promoter and, together with AtWRKY60, negatively regulate CRK5 expression.

It is speculated that the IIa WRKY transcription factor family of pumpkin has similar functions. IIb transcription factors have high homology with AtWRKY6.

AtWRKY6 can regulate the ageing process caused by salt stress in plants, suggesting that WRKys IIb in pumpkin have similar functions. Class III WRKY has high homology with AtWRKY46. Studies have shown that AtWRKY46 is regulated by ABA signalling and related to secondary rooting development under salt stress, so it is speculated that class II WRKys have similar effects. The classification results of the WRKY family according to the phylogenetic tree were also recorded in the pumpkin WRKY transcription factor information in Table 3.8.

Multi-sequence alignment of pumpkin CmWRKY

The most significant sequence structure of WRKY protein is the highly conserved "WRKYGQK" core sequence at the N-terminal and the variable zinc finger structure at the C-terminal. DNAMAN7.0 was used to conduct multiple sequence alignment for the full length of amino acid sequence of pumpkin WRKY transcription factor. The conserved domain sequences in the alignment results were marked and drawn with Weblogo as shown in Appendix C.

The figure shows that the WRKY conserved domain is highly conserved for pumpkin. WRKY I has two WRKY conserved domains. As can be seen from the figure, multiple sequence comparison results of two conserved domains at the C and N terminals of WRKY I can be seen. It is found that these two conserved domains are highly conserved and all contain WRKYGQK domain and C2H2 type zinc finger domain. However, the structure of zinc finger at N-terminal and C-terminal are slightly different. The difference between the N-terminal zinc finger domain and C-terminal zinc finger domain lies in the number of amino acid residues between the second cysteine (C) residue and the first histidine (H) residue, with 23 residues in the former and 24 in the latter. A highly conserved PRSYY sequence was found between WRKYGQK and zinc finger structure in the conserved domain of Class I WrKys. The conserved domains of IIa+b, IIc, IId and IIe only contain 1 WRKYGQK domain and 1 C2H2 type zinc finger domain. Most WrkygQKs in Class II have the same structure as those in Class I, but there are occasional variants. There were 5 amino acid residues between the two cysteine residues in the zinc finger structure of Class II, 1 more than that of class I, and 23 residues between the C and H residues. There were differences

between zinc finger domains in each subclass of class II. The III WRKY conserved domain contains a WRKY domain composed of WRKYGQK and a C2HC zinc finger domain composed of CX7CX23HXC.

Motif identification of CmWRKY in pumpkin

MEME was used to analyze the conserved momotif of the above 86 pumpkin WRKY transcription factors, as shown in Figure 3.12, 3.13. The modules are arranged

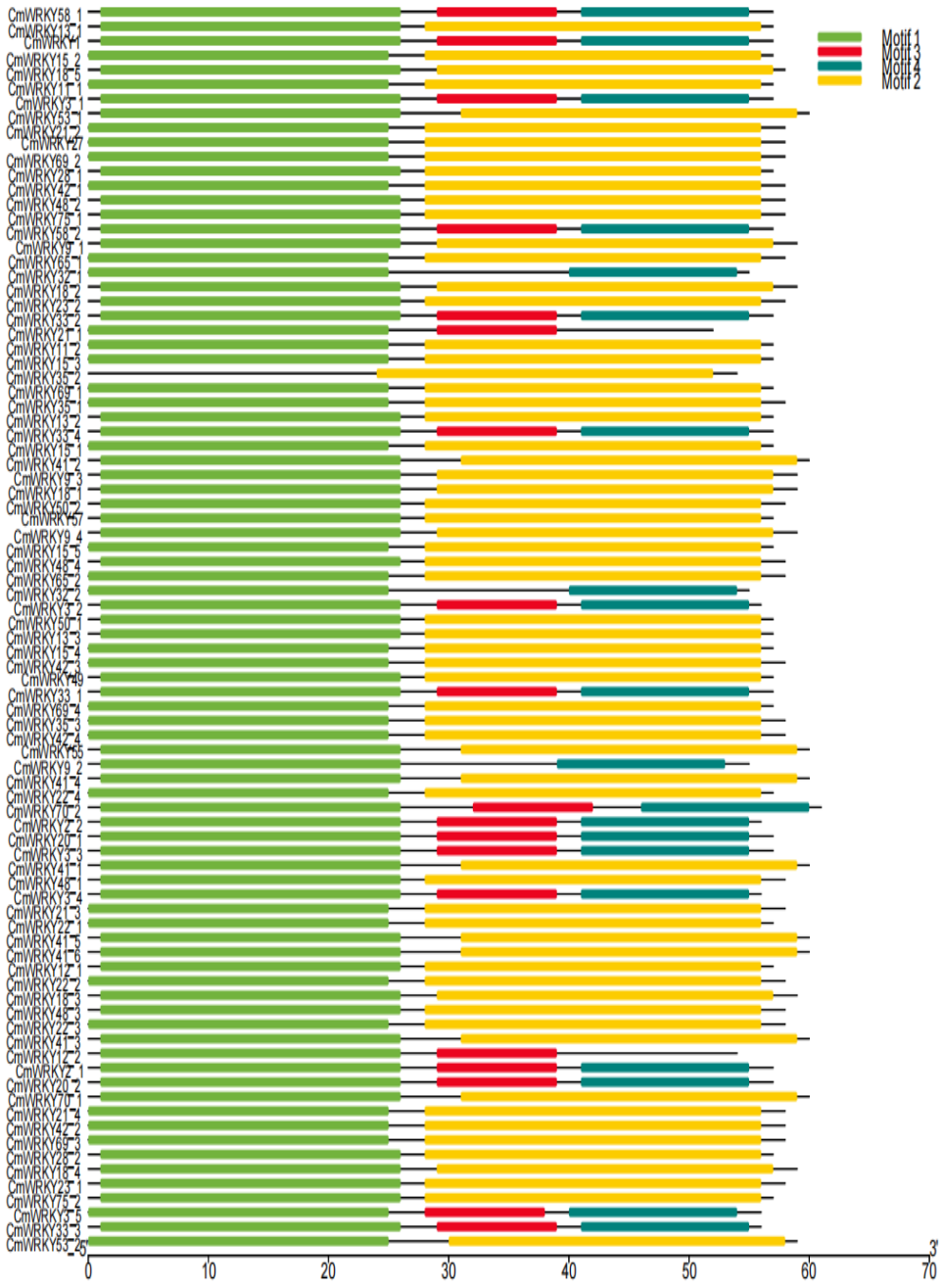


Fig.3.12. Model analysis (I) of the pumpkin WRKY transcription factor family

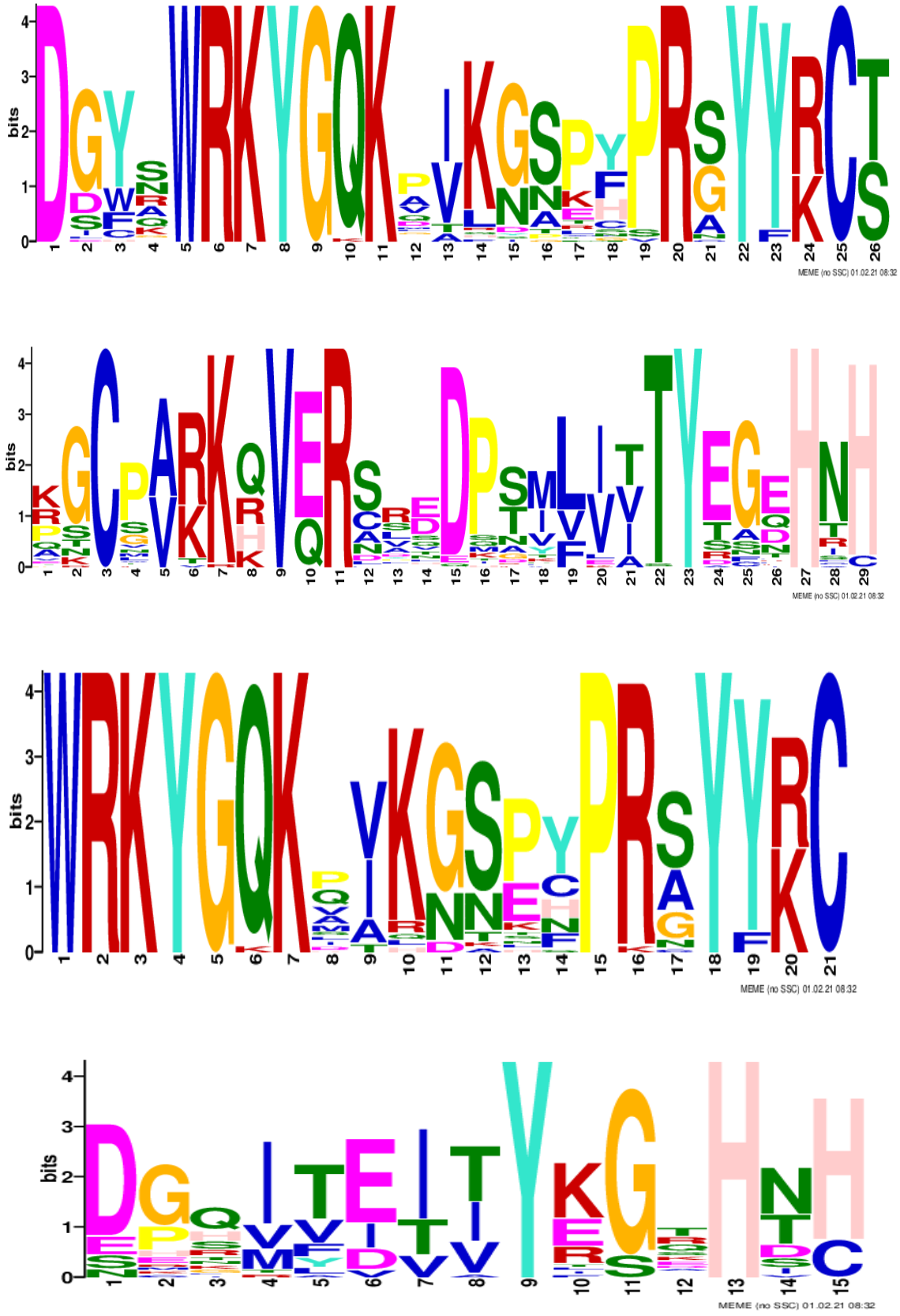


Fig.3.13. Model analysis (II) of the pumpkin WRKY transcription factor family

in ascending order by E value, and a small E value of a module indicates a high frequency of occurrence and a high homology. The amino acid lengths of the four motifs ranged from 11 (motif 3) to 38 (motif 2). It can be seen from the figure that all WRKY transcription factors have moduli 1, indicating that these moduli constitute homologous WRKY domains. The group I WRKY transcription factors also contain modules 3 and 4 representing the conserved WRKY domain at the C-terminal of group I. Meanwhile, group IIa and IIb both contain module 2, and group IId contains module 3. The same composition of modules in the same class of WrKys confirms the previous grouping results of pumpkin WrKys, and the appearance of a module in different classes of WRKY transcription factors indicates that they have similar functions.

Analysis of WRKY gene expression in salt mustard under salt stress

In order to further verify the reliability of transcriptome sequencing data, combined with gene family analysis and previous reports, we further removed the redundancy of 15 WRKY differentially expressed genes identified from transcriptome data. Twelve WRKY differentially expressed genes were screened and extracted from pumpkin leaf RNA treated with NaCl for 12h and 48h. The cDNA template was synthesized by reverse transcription and verified by qRT-PCR.

Real-time quantitative PCR showed that 10 genes were up-regulated and 2 genes down-regulated under NaCl treatment for 12h. Under NaCl treatment for 48h, 3 differentially expressed genes were up-regulated and 9 differentially expressed genes were down-regulated (Fig. 3.14). Although the results of qRT-PCR analysis were not exactly the same as the results of sequencing, the differential expression trend was the same, which might be related to the differences between the two analysis techniques and methods, and could also prove the correctness and reliability of the results of transcriptome data.

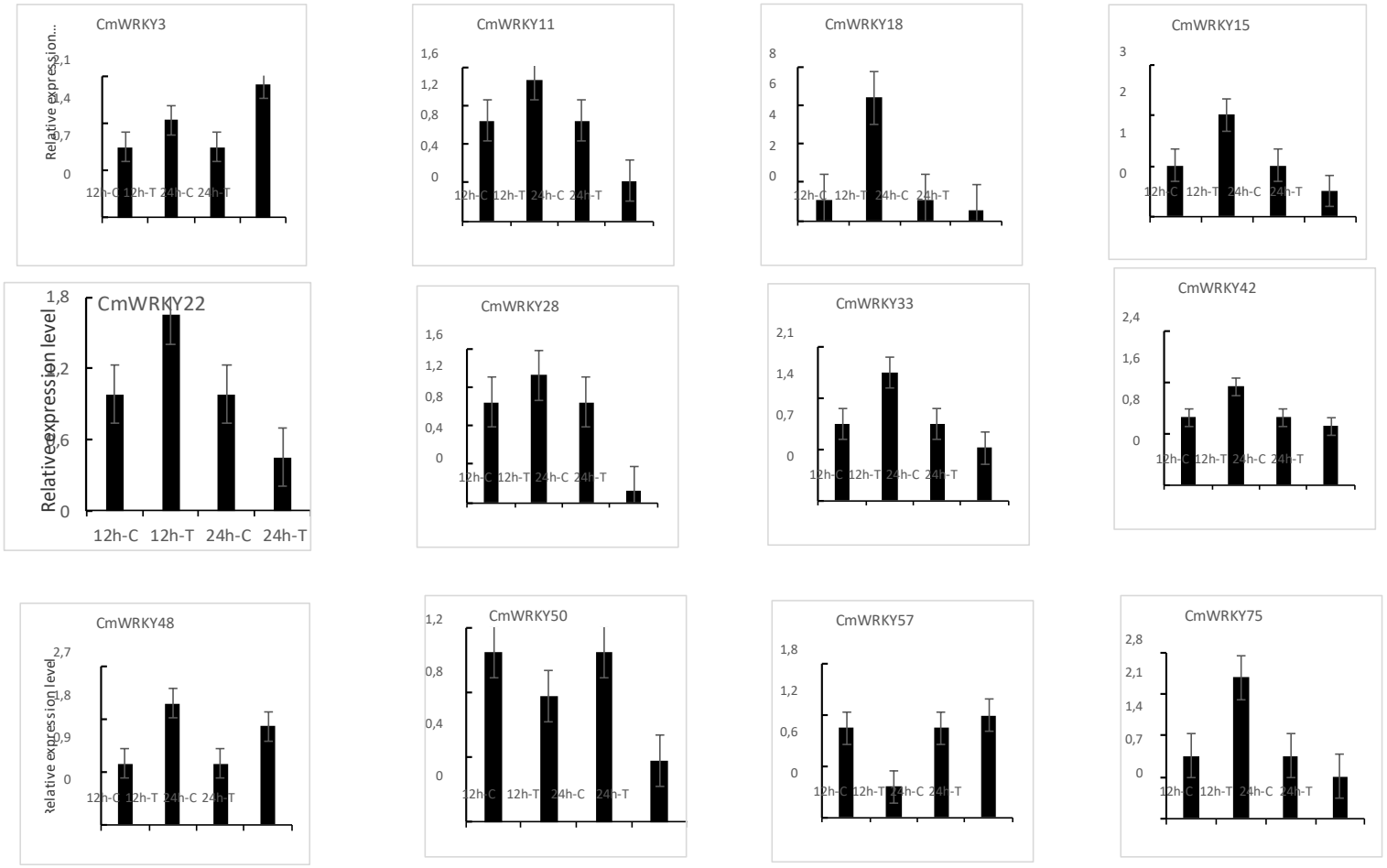


Fig. 3.14. Validation annlysis of the relative expression level of CmWRKYs by qRT-PCR

WRKY transcription factors are a class of transcription regulatory factors unique to higher plants, as well as indispensable regulatory hubs in plant life activities, which are involved in plant growth and development and various biological and abiotic stress response processes. Currently, the role of several WRKY family transcription factors in abiotic stress response has been demonstrated in Arabidopsis thaliana [333], cotton [334], rice [335], soybean [336] and other species. In this study, we analysed the transcriptome data of pumpkin control and salt treatment, and used iTAK software and other methods to predict and screen 12 WRKY family transcription factors that were induced or inhibited by salt. Sequence analysis showed that all of them had core conserved sequences of WRKY family genes, which proved the reliability of our predictive screening method.

Through sequence alignment and phylogenetic analysis, it was found that CmWRKY3 and CmWRKY33 had the highest homology among the 12 WRKY transcription factors, which were distributed in the phylogenetic branch of type I WRKY family (containing two WRKY domains). In Arabidopsis thaliana, the functions of several genes in this clade have been shown to be related to plant stress response. For example, AtWRKY3 and AtWRKY4, which have the highest homology with CmWRKY3 and CmWRKY33, both play a role in disease resistance of Arabidopsis thaliana [337]; AtWRKY20 and AtWRKY26 were associated with the response of jasmonic acid (JA) [338]; AtWRKY25, AtWRKY26 and AtWRKY33 were associated with temperature stress response [339]. The type II WRKY family (containing one WRKY core domain and one C2H2 motif) consists of five subfamilies of GroupIIa to e. In this study, 10 transcription factors belong to type II, among which CmWRKY18 and CmWRKY42 are located in GroupIIa and GroupIIb. CmWRKY48/50/57/75 is located in the GroupIIc subfamily. Documented studies have shown that Transcription factors of these three subfamilies are involved in plant embryo development, ageing, and substance transport [340], drought [341], salt stress [342], disease defence, and stress hormone response [343] and other aspects also play an important role. Therefore, it is speculated that WRKY transcription factors isolated in this study may also be involved in the stress response of pumpkin.

In the process of plant evolution, in order to adapt to environmental changes and various biological and abiotic stresses, plants have formed a set of their own adaptive mechanisms. Under stress conditions, plants should not only respond quickly to short-term stimuli from internal and external environmental signals, but also initiate a series of protection systems to cope with long-term adversity stress [344]. Changes in the temporal and spatial expression patterns of transcription factors play a key role in regulating plant response to stress. Tissue specific expression analysis of 12 target WRKY transcription factors showed that most of the genes had typical tissue specific expression. Normally, gene expression localization is compatible with its function. For example, maize GmGRP3 gene is specifically expressed in roots and controls root development [345]. MtSERK1 gene was specifically expressed in embryos and correlated with embryo development [346]. Therefore, it is speculated that the differences in the temporal and spatial expression patterns of these WRKY transcription factors may be related to their functional diversity in response to salt stress [347].

In conclusion, in this study, we screened 12 WRKY family transcription factors in pumpkin in response to salt stress, and analysed their phylogenetic relationships, spatio-temporal expression patterns, tissue-specific expression characteristics, and transcriptional activities under salt stress in detail. However, there are still some unsolved problems. Such as: do these genes have tolerance function? If such a function exists, what is the molecular mechanism of their involvement in hexagrams stress response? In addition to salt stress, are they involved in other stress response or growth regulation processes? And so on. In the future, further studies should be carried out by constructing overexpressed or functionally deficient transgenic strains to further reveal their functions and molecular regulatory mechanisms.

3.4.2. An efficient transient transformation system for gene function studies in pumpkin

Transient transformation is a promising tool for the study of gene function. Here, an efficient Agrobacterium-mediated transient transformation system was developed for gene function studies in pumpkin.

To optimize the conditions for highly efficient transformation, experiments on the time effect of sonication and vacuum infiltration were carried out. As treatment time increased, the efficiency was gradually enhanced. Post-infiltration followed by 3 days co-culture, seeds without the GUS assay were planted in soil for further use. However, the damage to seedlings caused by sonication and vacuum infiltration also increased. So we carried out damage assessment and determined the optimal treatment time, 120 s sonication and 5 min vacuum infiltration. To apply this transformation method to gene function analysis, we tested it by using a salt tolerance gene, named StNHX1 and cloned in wild aubergine previously [348].

To detect the function of StNHX1 in pumpkin, post-co-cultured seeds were planted in pot and applied with 200 mmol/L NaCl solution for several days. The results showed that over-expression of StNHX1 improved salt tolerance for pumpkin seedlings. Morphologically, the cotyledons in StNHX1 were green with slight wilting, while the control cotyledons turned yellow and withered rapidly. Compared with control plants, the fresh weight of StNHX1 seedlings was higher (Fig. 3.15).

All the 5 genotypes showed relatively higher resistance to salt stress when the StNHX1 gene was over-expressed. Except for ‘112−2’, the other 4 genotypes displayed significant differences compared to the controls in fresh weight. The fresh weight of root in 4 genotypes was significantly higher than the control, except for ‘009− 1’ (Fig. 3.16).

Since the method of agroinjection for subcellular localization of a CmPHD1 protein is efficient in analysing gene expression, we carried out experiments to determine the expression of GFP, which is widely used as a tag for subcellular localization (Fig. 3.17). Initially, GFP fluorescence was detected using a special excitation light source. It showed that EGFP was expressed in some parts of cotyledon.

The results showed that about 7-day-old seedlings with dark green, fully flat cotyledon and one true leaf are best for subcellular localization analysis (Fig. 3.18). To further test this method, we analysed subcellular localization of a pumpkin protein searching from the Cucurbit Genomics Database (http://cucurbitgenomics.org/). The gene ‘CmoCh05G005550’ encodes a zinc finger like PHD-finger protein, named

CmPHD1. The subcellular localization of this protein - the nucleus (http://www.csbio.sjtu.edu.cn/bioinf/ plant-multi/) [349].

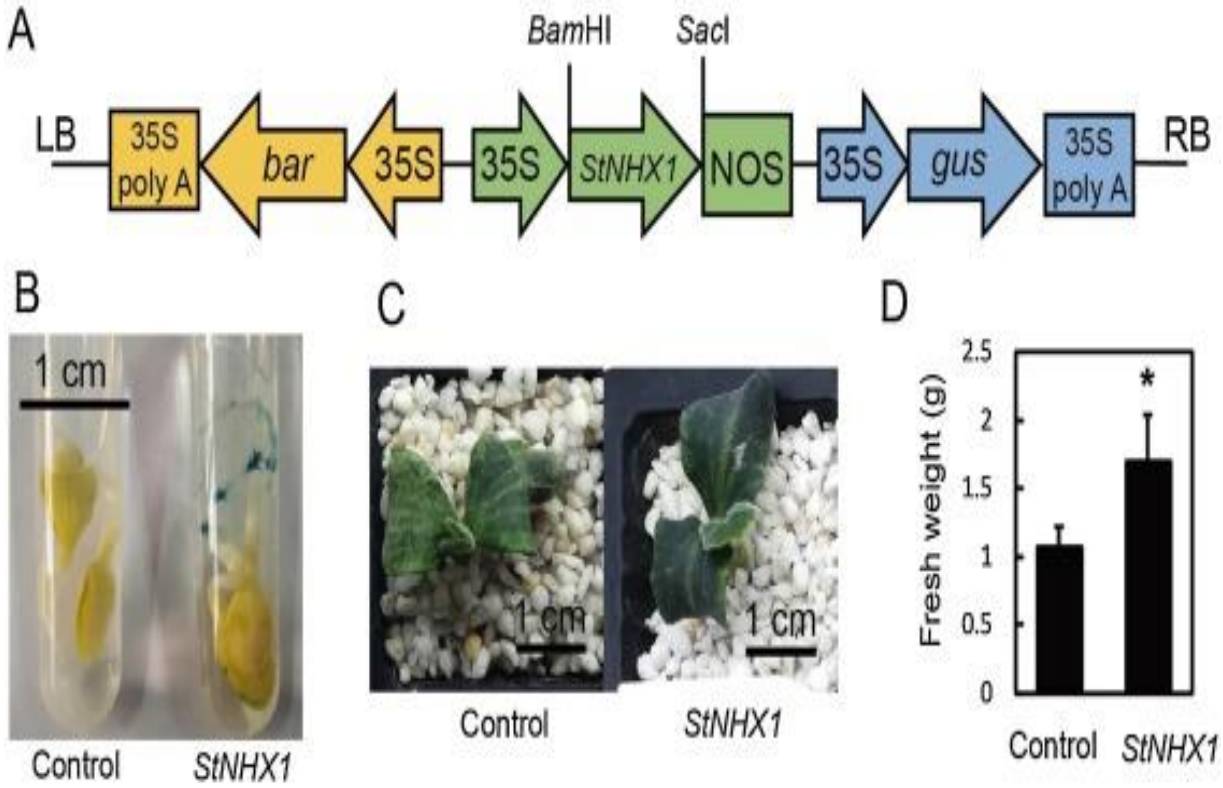


Fig. 3.15. Over-expression of *StNHX1* increases salt tolerance in young seedlings *Agrobacterium* harbouring empty vector pCAMBIA1302 (without *GUS* gene) was used as control. The plant material is '360−3'. A: Schematic diagram of T-DNA region of pCAMBIA3301-*StNHX1* plasmid; B: GUS staining assay. C: Morphology of young seedlings after salt stress treatment; D: Comparison of fresh weight after salt stress treatment, * indicates difference which is statistically significant at the $P < 0.05$ level. The results of 3 independent experimental replicates are shown.

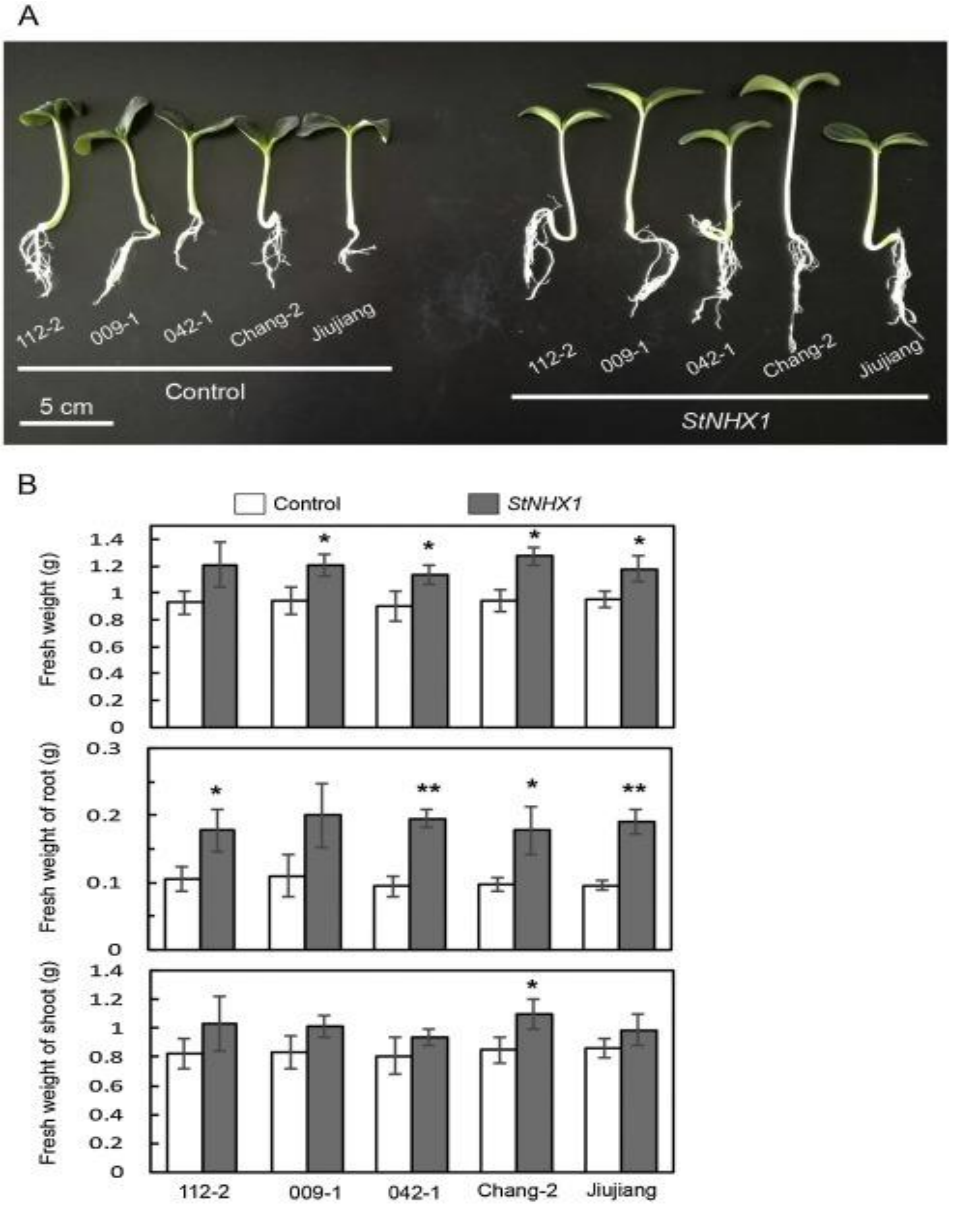


Fig. 3.16. Over-expression of ***StNHX1*** increases salt tolerance in 5 pumpkin genotypes**.**

Agrobacterium harbouring empty vector pCAMBIA1302 was used as a control. A: Morphology of young seedlings after salt stress treatment. The picture was taken 3~5 days post-treatment; B: Comparison of fresh weight after salt stress treatment, * and ** indicate differences that are statistically significant at the $P < 0.05$ and $P < 0.01$ levels, respectively.

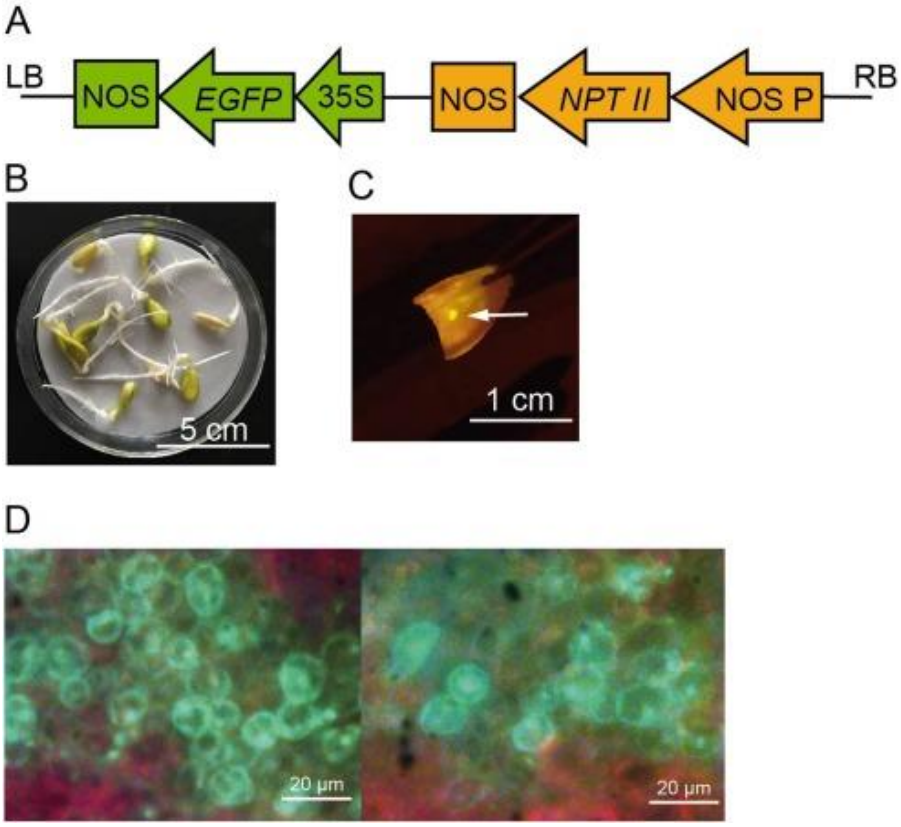


Fig. 3.17. GFP detection of mesophyll cells in cotyledons.

A: Schematic diagram of T-DNA region of the vector pBI121-*EGFP*. B: Seeds of '360−3' after cultivation in a petri dish. C: Fluorescent detection of cotyledon. The white arrow indicates the part of green fluorescence. D: Green fluorescence of mesophyll cells in two fields under a microscope, wherein the red part is chlorophyll auto-fluorescence.

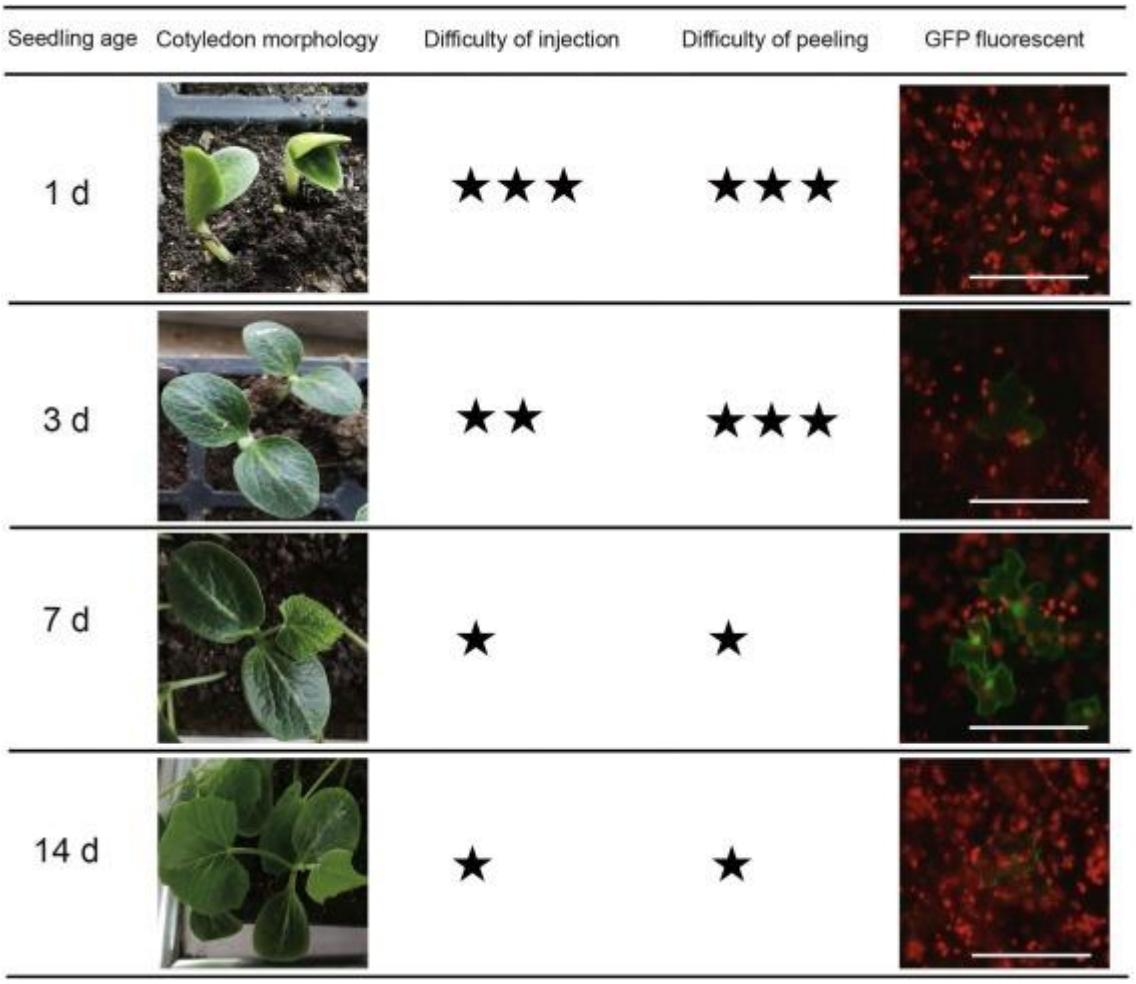


Fig. 3.18. Optimization of experimental conditions for GFP detection.
The plant materials are '360−3'. *Agrobacterium* harbouring pBI121-*EGFP* was used for infection. GFP fluorescence was detected 3 days post-injection. The red dots indicate chlorophyll auto-fluorescence. Three stars, two stars and one star stand for 'difficult', 'slightly difficult' and 'easy'.

The fluorescent signals were detected through DAPI and GFP excitation light under a microscope. Strong density of green fluorescence from free GFP was detected in the nucleus, cytoplasm and cell membrane (Fig. 3.19). The CmPHD1-EGFP fusion protein was detected in the nucleus of cotyledon epidermal cells, where it showed green fluorescence.

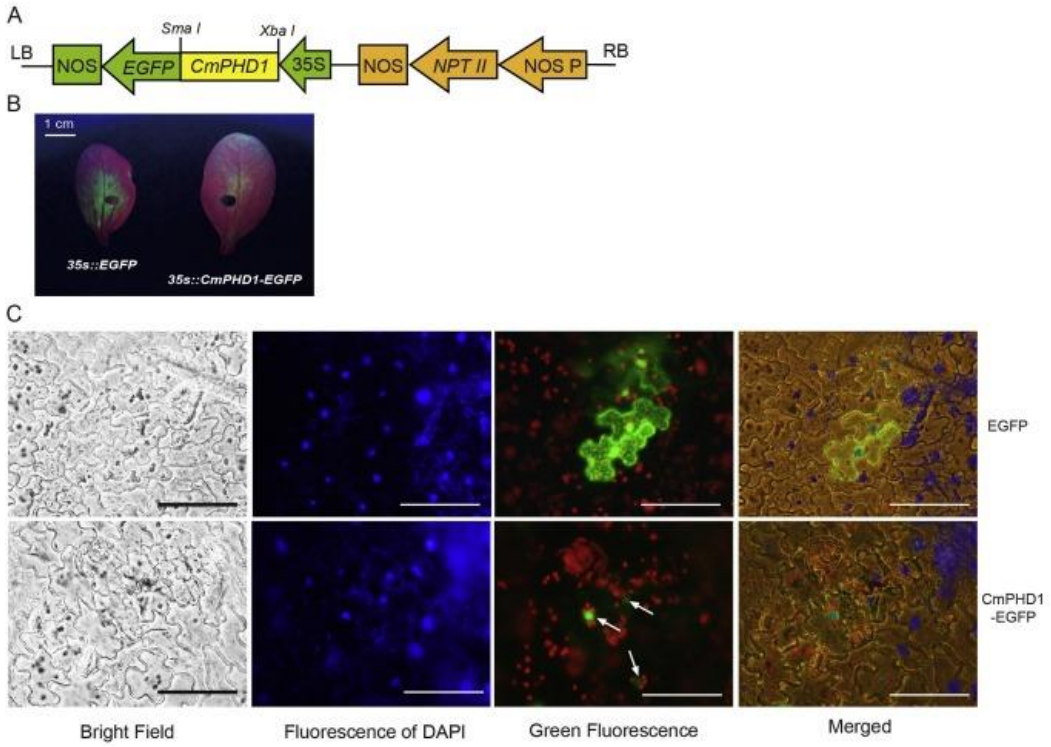


Fig. 3.19. Subcellular localization of the CmPHD1-EGFP fusion protein.

The plant material used was '360−3'. *Agrobacterium* harbouring pBI121-*EGFP* and pBI121-*CmPHD1-EGFP* were used for infection, respectively. A: Schematic diagram of the T-DNA region of the vector pBI121-*CmPHD1-EGFP*. B: Detection of GFP fluorescence using a special excitation light source. C: CmPHD1-EGFP fusion proteins were localized to the nucleus of epidermal cells. The DAPI fluorescent is an indicator of the nucleus. The white arrows indicate green fluorescence of the CmPHD1-EGFP fusion protein.

In this study, we improved the transformation efficiency by using sonication and vacuum infiltration in pumpkin. The optimal treatment time and intensity were confirmed. A salt-tolerant gene, StNHX1, was transiently over-expressed in roots and cotyledons of pumpkin using this method. (Figs. 3.15, 3.16).

We have developed an efficient transient transformation system for the study of gene function and protein subcellular localization in pumpkin. The detailed experimental procedure is summarized in Fig. 3.20.

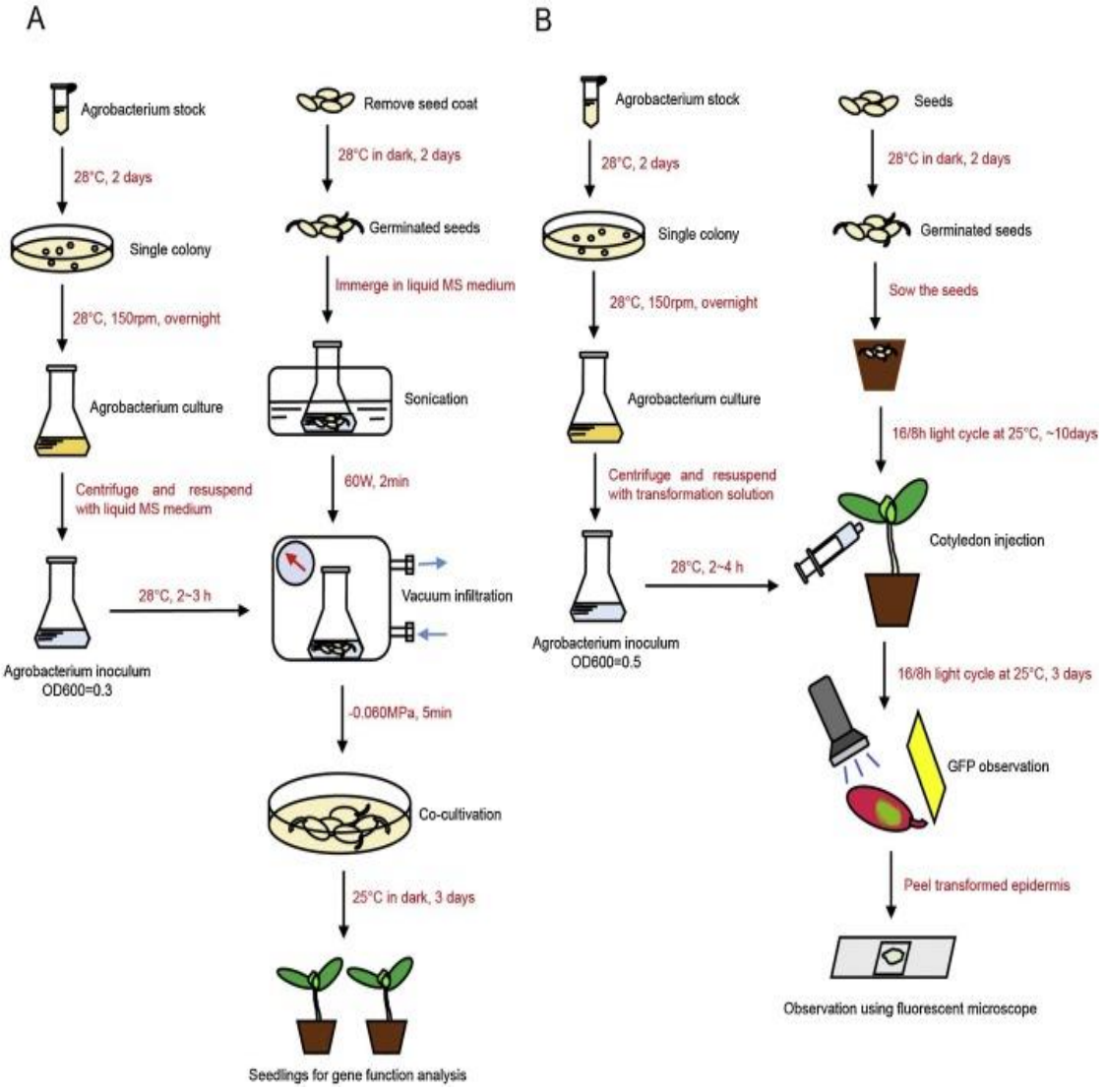


Fig. 3.20. Procedure of transient transformation.

A: Procedure of transient transformation via agroinfiltration for gene function analysis.

B: Procedure of transient transformation via agroinjection for protein subcellular localization.

This method has some advantages compared with the current transient transformation methods:

a) The subcellular localization of a pumpkin protein is more convincing than using model plants.
b) It is time-saving because the whole experiment only takes about 14 days.
c) It is cost-effective because it does not require expensive equipment, such as devices for particle bombardment and a laser confocal microscope.
d) It is easy to peel cotyledon epidermis to observe a single layer of living cells.
e) It is easy to operate because cotyledon is thick and is easy to inject.
f) The chlorophyll content of epidermal cells is low, resulting in less interference by chlorophyll auto-fluorescence

We expect this technique could be widely applicable to study gene function on *Cucurbita* crops in the future, including for establishing mechanisms of response and adaptation to salt stress.

3.5. Plant grafting and interspecies interactions in the system of measures to ensure adaptation and increasing salt tolerance of plants

3.5.1. Eco-physiological aspects of the use of salt-resistant pumpkin rootstocks during the cultivation of *Cucurbitaceae*

Photosynthesis

PN: The PN of the grafted-root line was higher than that of corresponding own-root line during fruit development stage, and the difference between grafted-root line and corresponding own-root line increased with prolongation of fruit development time in both diploid watermelon and triploid watermelon (Fig. 3.21) [246]. These results indicate that pumpkin rootstock could improve photosynthesis of diploid and triploid watermelon.

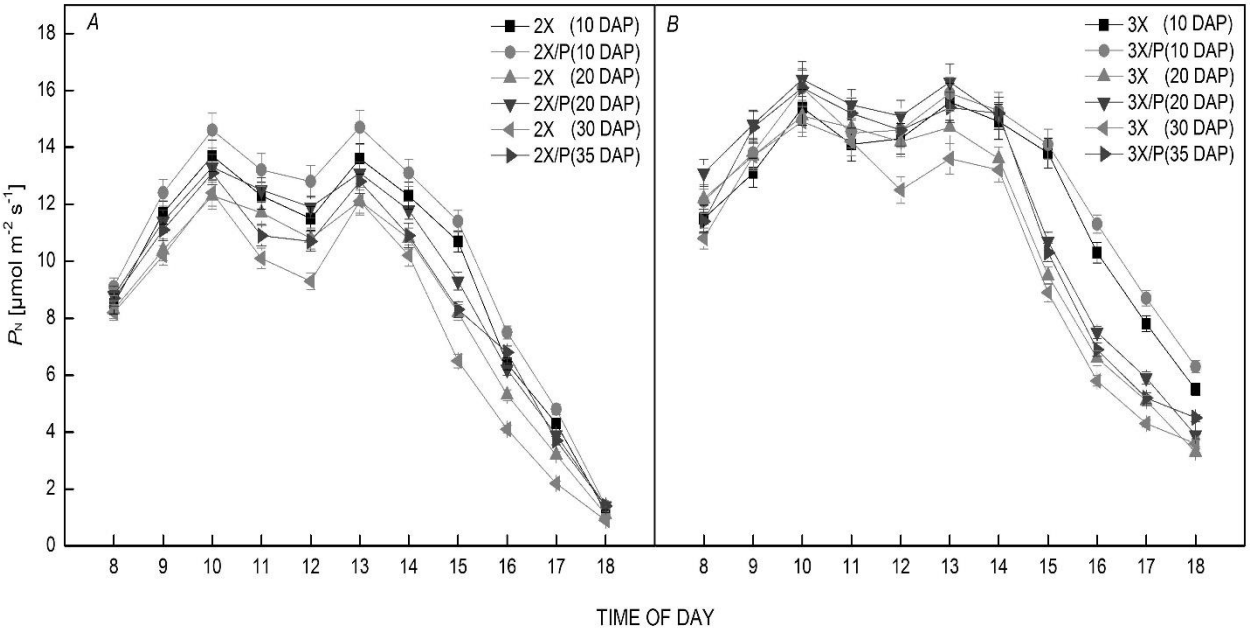


Fig. 3.21. Changes of PN in grafted-root watermelon lines and own-root watermelon lines during fruit development stage

Chl fluorescence parameters

During fruit development stage, the Fv/Fm, ΦPSII and ETR of the grafted-root line were higher than those of corresponding own-root line regardless of diploid watermelon or triploid watermelon (Fig. 3.22). The Fv/Fm, ΦPSII and ETR showed significant differences ($P<0.05$) between grafted-root line and own-root line during fruit development stage in diploid watermelon (Fig. 3. 22ADE). Meanwhile, the Fv/Fm, ΦPSII and ETR showed significant differences ($P<0.05$) between grafted-root line and own-root line during late stage (30DAP) of fruit development in triploid watermelon (Fig. 3.22BCF).

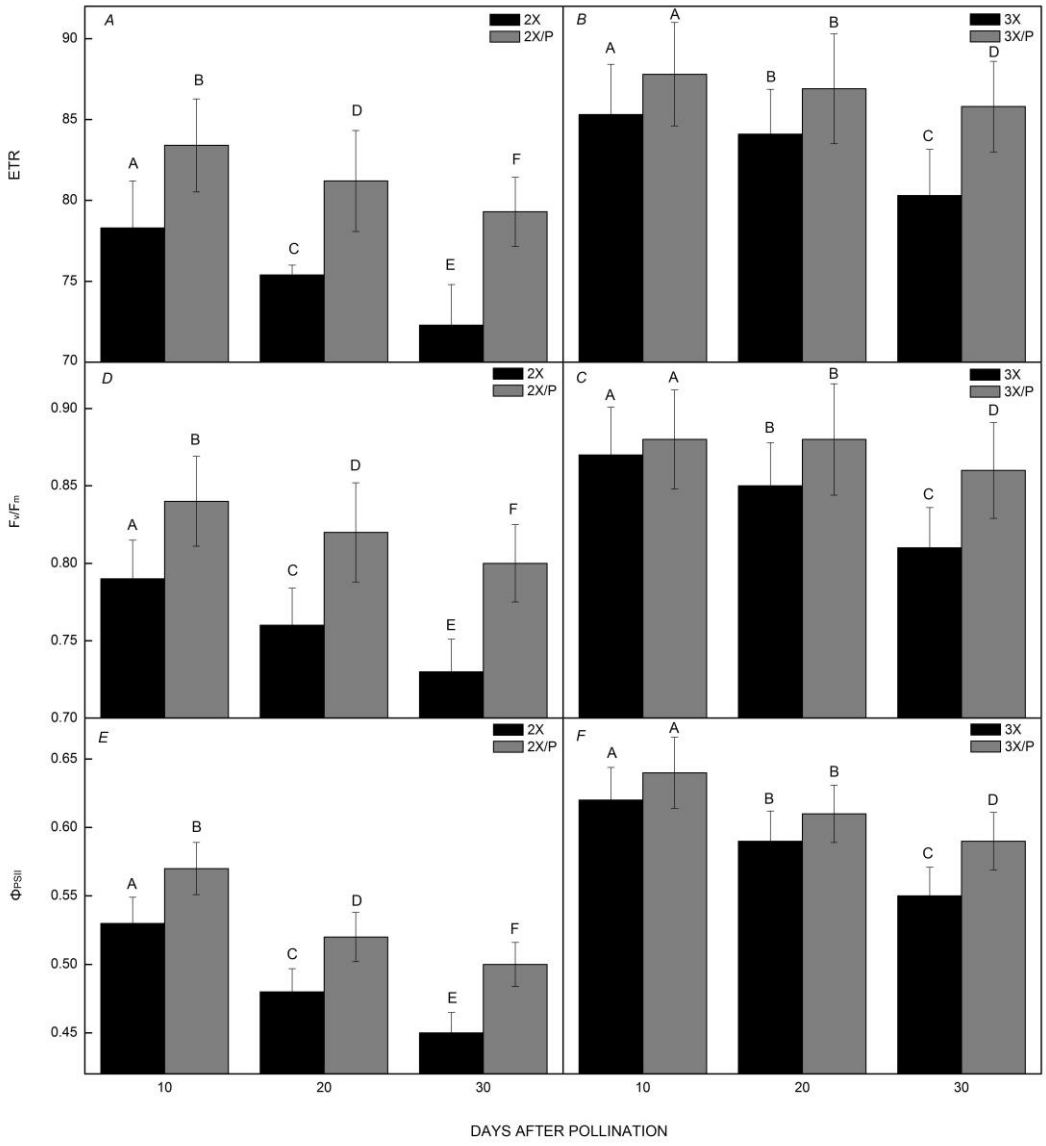


Fig. 3.22. Changes of chlorophyll fluorescence parameters in grafted-root watermelon lines and own-root watermelon lines during fruit development stage

The activity of alkaline α-galactosidase

The activity of alkaline α-galactosidase in fruits decreased with the prolongation of fruit development time for all watermelon lines. However, alkaline α-galactosidase activity of own-root line decreased sharper than that of corresponding grafted-root line in both diploid watermelon and triploid watermelon. For diploid watermelon, the alkaline α-galactosidase activity in fruits of grafted-root line was significantly ($P<0.05$) higher than that in fruits of own-root line during fruit development stage

(Fig. 3.23A). For triploid watermelon, the activity of alkaline α-galactosidase was higher in the grafted-root line than that in own-root line during fruit development stage, and the difference of alkaline α-galactosidase activity between grafted-root line and own-root line was significant (P<0.05) in late development stage (30 DAP) (Fig. 3.23B).

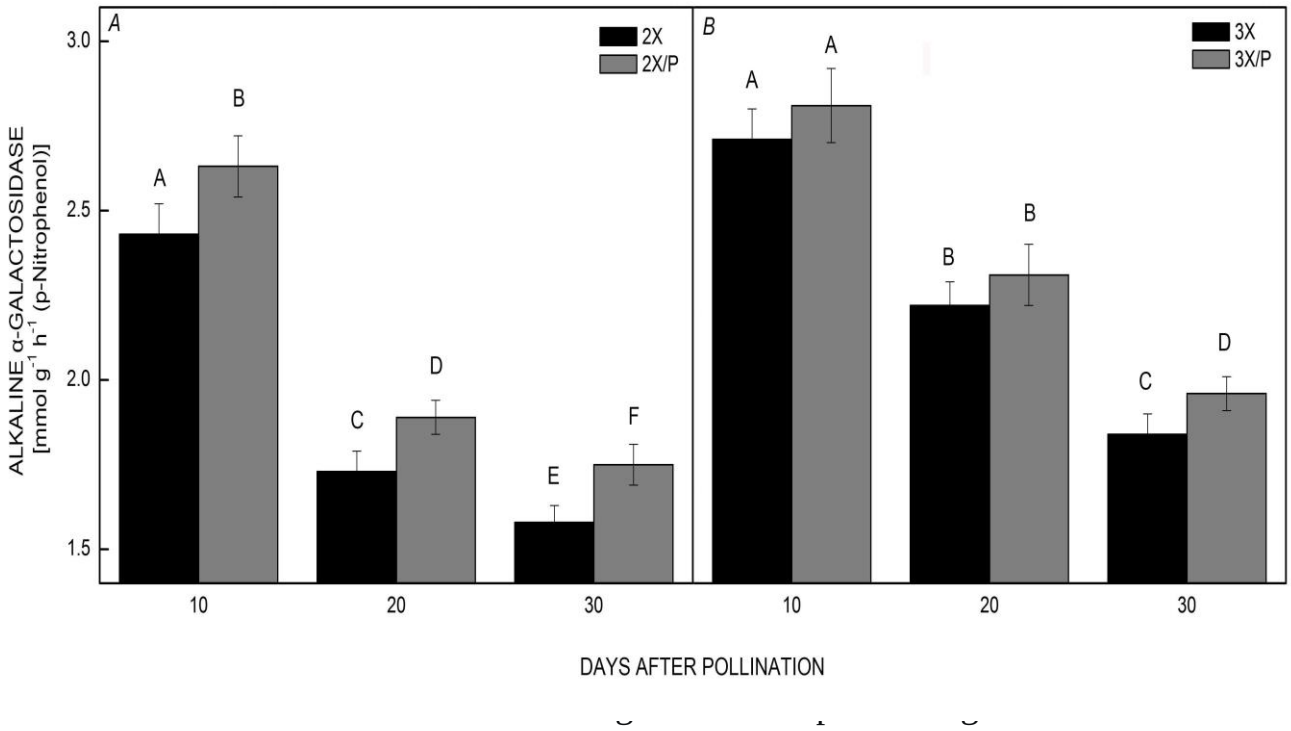


Fig. 3.23. Alkaline α-galactosidase activity in fruits of grafted-root watermelon lines and own-root watermelon lines during fruit development stage

Dry matter and mass accumulation of fruit

The changes in dry matter accumulation were similar to the changes in mass accumulation during fruit development for all watermelon lines (Fig. 3.24). Single fruit mass and dry matter content increased with prolongation of fruit development time for all watermelon lines. For diploid watermelon, the single fruit mass and dry matter content of grafted-root line were significantly (P<0.05) higher than those of own-root line during fruit development (Fig. 3.24AD).

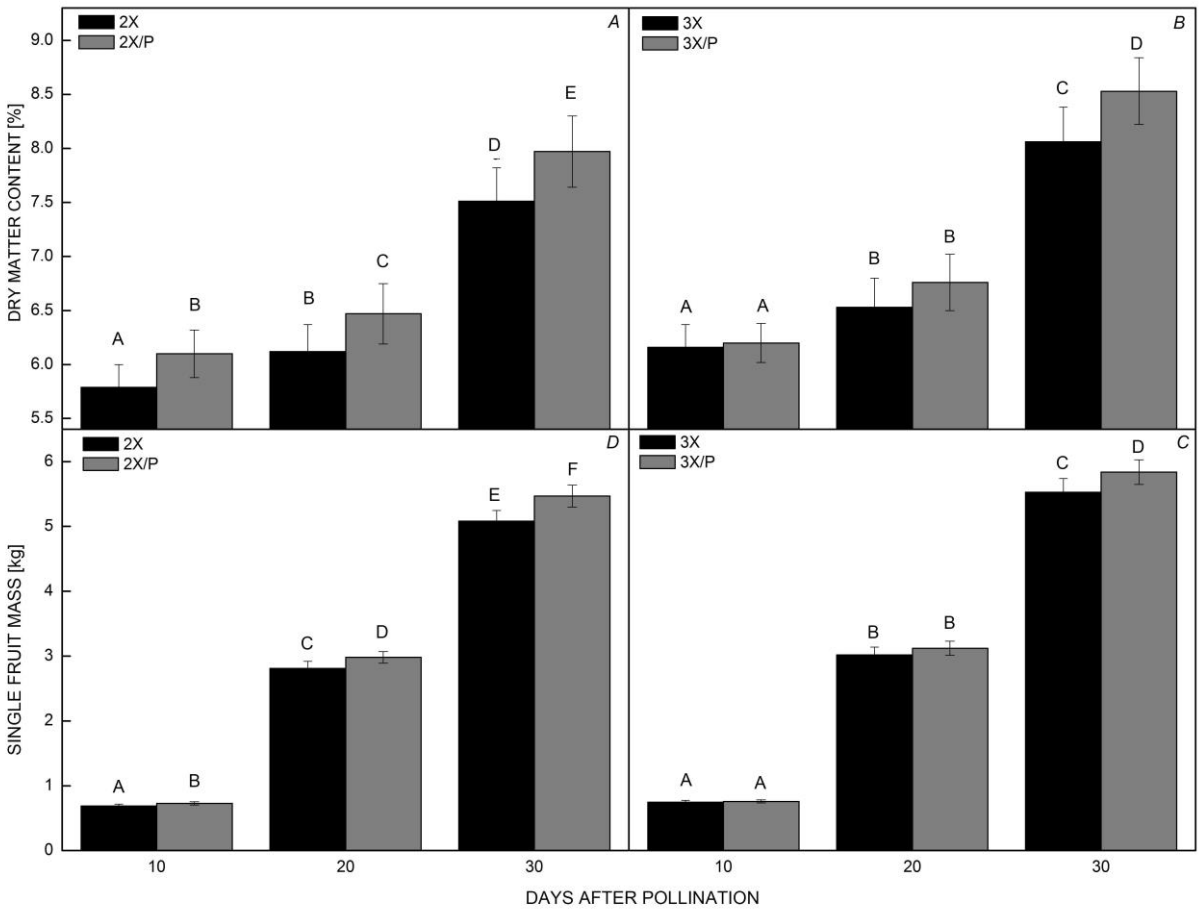


Fig. 3.24. Fruit mass and dry matter accumulation in grafted-root watermelon lines and own-root watermelon lines during fruit development stage

For triploid watermelon, the single fruit mass and dry matter content of grafted-root line were higher than those of own-root line at various stages of fruit development, and the difference was significantly ($p<0.05$) during late stage (30 DAP) of fruit development (Fig. 3.24BC).

The activities of IAI, SPS and SuSy

For diploid own-root line and corresponding grafted-root line, the IAI activity in flesh first increased sharply and then declined slightly, SuSy activity in flesh first declined slightly and then increased sharply, and SPS activity in flesh increased all the time during fruit development stage (Fig. 3.25ADE).

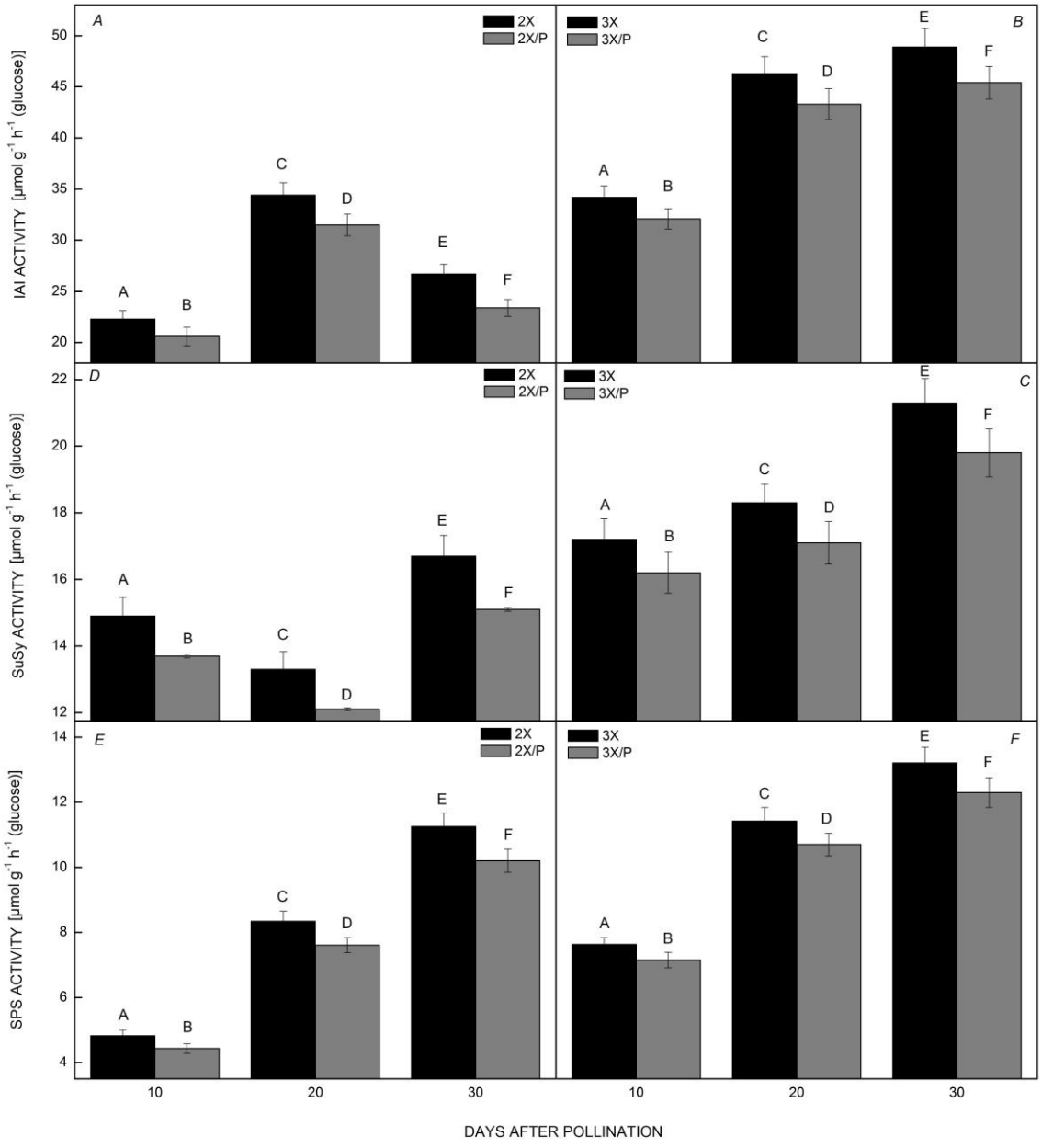


Fig. 3.25. The activities of IAI, SPS and SuSy in flesh of grafted-root watermelon lines and own-root watermelon lines during fruit development stage

Meanwhile, the activities of IAI, SuSy and SPS in flesh increased with the prolongation of fruit development time in triploid own-root line and corresponding grafted-root line (Fig. 3.25BCF). Both diploid watermelon and triploid watermelon, the activities of IAI, SPS and SuSy were significantly ($P<0.05$) lower in grafted-root line than those in corresponding own-root line during fruit development stage.

Sucrose and total sugar accumulation

The changes in total sugar accumulation were similar to the changes in sucrose accumulation during fruit development for all watermelon lines (Figure 3.26ABCD). The contents of sucrose and total sugar in flesh increased with prolongation of fruit development time for all watermelon lines (Figure 3.26ABCD). The sucrose and total sugar contents in flesh of grafted-root line were significantly ($P<0.05$) lower than those in flesh of own-root line, regardless of diploid watermelon or triploid watermelon.

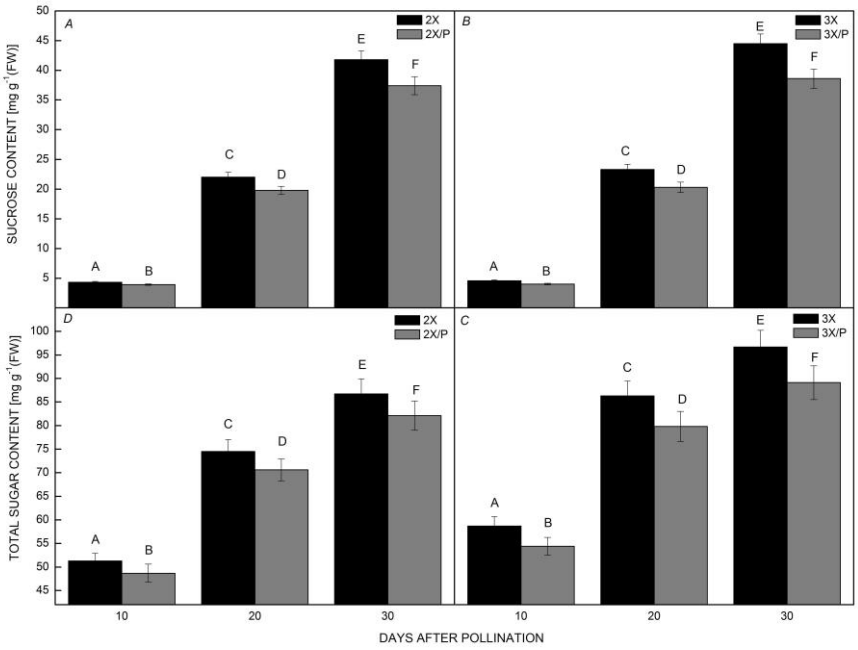


Fig. 3.26. Sucrose and total sugar accumulation in flesh of grafted-root watermelon lines and own-root watermelon lines during fruit development stage

Mass and sucrose accumulation of crop fruit depend on the capacity of source tissues (especially leaves) to produce photoassimilates, during the fruit development process, as well as on the ability of sink tissues to unload this photoassimilates. Photosynthesis is the physico-chemical process to transduce light energy into chemical energy, and it provides the organic blocks for plant growth and development. Theoretically, yield and sugar content of crop fruit can be increased by promoting photosynthesis because photosynthesis is the major metabolic pathway that converts

carbon dioxide (CO_2) into organic compounds, such as fructose, glucose, sucrose, and starch in the plant. Chl fluorescence provide a measure of the functional status of photosynthetic and have been used as sensitive indicators of plant photosynthetic performance. The increase in Fv/Fm, ETR and ΦPSII indicates a progressive up-regulation in the quantum yield of photosynthesis, rates of carbon fixation and photosynthetic efficiency. The PN, ETR, Fv/Fm and ΦPSII were higher in rootstock-grafted line than in corresponding own-root line during the fruit development stage in both diploid watermelon and triploid watermelon. These results indicate that pumpkin rootstock could improve photosynthetic capacity and efficiency of light energy utilization, which could improve capacity of leaves to produce photoassimilates in rootstock-grafted watermelon line. Raffinose oligosaccharides are the main photoassimilates that are translocated in the phloem of Cucurbitaceae family members. Alkaline α-galactosidase is an important enzyme for raffinose oligosaccharides unloading and partitioning in cucumber and melon sink tissue. Pumpkin rootstock improved alkaline α-galactosidase activity in both diploid watermelon line and triploid watermelon line. More photoassimilates could be allocated to grafted-root watermelon fruit than to corresponding own-root watermelon fruit. Pumpkin rootstock could improve synthesis of photoassimilates and their unloading into fruit in both diploid watermelon and triploid watermelon. This may be the reason why pumpkin rootstock can increase fruit mass and dry matter content in both diploid watermelon and triploid watermelon.

Sucrose, glucose and fructose are the main sugars in watermelon. Sucrose increase rapidly during fruit developmental stage, while fructose and glucose contents are almost constant during watermelon fruit development. Therefore, the difference of sugar content among watermelon varieties is mainly determined by sucrose content. The sugar accumulation of crop depend on the ability of sink tissues to convert photoassimilates into sugar. The conversion capacity of photoassimilates to sucrose has become an important factor for sugar accumulation of watermelon fruit. The most well-studied enzymes that function in sucrose metabolism during fruit development include three enzyme families, i.e., insoluble acid invertase (IAI), sucrose synthase (SuSy) and sucrose phosphate synthase (SPS). IAI is an extracellular enzyme that is

bound to the cell wall. Both phloem unloading and sucrose translocation to the developing sinks require IAI in sucrose-translocating plants. Sucrose synthase (SuSy) is a key enzyme that catalyses the synthesis of sucrose. A positive correlation between SuSy activity and sucrose accumulation was also reported in melon fruit and watermelon, suggesting that this enzyme plays an important role in determining sugar accumulation in sweet cucurbit fruit. Sucrose phosphate synthase (SPS) is another key enzyme that catalyses sucrose synthesis. SPS is localized in the cytosol of the cells of many tissues, including sink organs such as seeds and fruit. SPS activity is positively related to sucrose accumulation in melon and watermelon. In this experiment, although pumpkin rootstock increased PN, ETR, Fv/Fm, ΦPS and alkaline α-galactosidase activity, it decreased activities of IAI, SPS and SuSy. The lower activities of IAI, SPS and SuSy would reduce conversion of photoassimilates to sucrose in grafted-root watermelon line. Sucrose accumulation was down-regulated in the grafted-root line versus the corresponding own-root line in both diploid watermelon and triploid watermelon. As a result, sucrose and total sugar contents of grafted-root line were lower than those of corresponding own-root line in both diploid watermelon and triploid watermelon.

In conclusion, pumpkin rootstock increased PN, ETR, Fv/Fm and ΦPSII during fruit development stage in both diploid watermelon line and triploid watermelon line. These advantages could improve photosynthetic capacity, utilization efficiency of light energy, and photosynthate assimilation in both diploid watermelon line and triploid watermelon line. The activity of alkaline α-galactosidase was much higher in grafted-root watermelon line than in corresponding gown-root watermelon line during fruit development, which accelerated photoassimilate unloading and partitioning in grafted-root watermelon fruit. It was the reason why the dry matter content and mass of grafted-root line were higher than those of corresponding own-root line in both diploid watermelon fruit and triploid watermelon fruit. In the other hand, pumpkin rootstock decreased the activities of IAI, SPS and SuSy during fruit development in both diploid watermelon flesh and triploid watermelon flesh. The lower IAI, SPS and SuSy activities were disadvantageous to converting photoassimilate into sucrose in grafted-root watermelon lines, which made the

sucrose and total sugar contents in flesh of grafted-root line were lower than those in flesh of corresponding own-root line regardless of diploid watermelon or triploid watermelon.

3.5.2. Eco-physiological aspects of use of mycorrhizal fungi

In this experiment, the commercial pumpkin rootstock cultivar ‘Baimi112’ (Cucurbita maxima), the commercial triploid watermelon cultivar ‘Zheng No.3 (3X)’ and the corresponding diploid watermelon line ‘Zheng No.3 (2X)’ [Citrullus lanatus (Thunb.) Matsum. and Nakai.] were used. The average colonisation of Rhizophagus intraradices (AMF) was 95.6% and the average spore density was 549 per 10 g of air-dried soil. The spores, mycelium, colonised root fragments, and dried sand-soil were mixed to use as AMF inoculums. The treatments were: (1) well-watered and non-AMF (WW); (2) well-watered and inoculated with AMF (WW + AMF); (3) deficit irrigation and non-AMF (DI); (4) deficit irrigation and inoculated with AFM (DI + AMF) [313].

Deficit irrigation was accompanied by an increase in soil solution concentrations and, accordingly, the creation of conditions inherent to salt stress. Accordingly, the presented research allows us to evaluate the role of interspecies interactions in the adaptation of plants to salt stress.

Arbuscular mycorrhizal fungi are able to colonise and establish symbiotic mutually beneficial associations with the roots of most agricultural crops and increase the effective absorptive area of the roots [350-352]. In this study, the AMF improved the RWC, PN and the activities of the alkaline α-galactosidase, IAI, SuSy and SPS under both the WW and DI, which was accompanied by an increased concentration of salts in the soil solution. As a result, AMF contributed to improving the yield and sugar content of the watermelon fruit (Tables 3.9–3.11, Appendix D).

Table 3.9. Alkaline α-galactosidase activity [mmol/g (p-nitrophenol)·h] under different treatments during the fruit[1]

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
Alkaline α-galactosidase	10 DAP	WW	2.43 ± 0.09	2.62 ± 0.11	2.64 ± 0.09	2.86 ± 0.12
		WW + AMF	2.48 ± 0.10	2.71 ± 0.10	2.71 ± 0.11	2.97 ± 0.10
		DI	1.59 ± 0.06	1.91 ± 0.07	1.82 ± 0.07	2.11 ± 0.08
		DI + AMF	1.82 ± 0.08	2.52 ± 0.09	2.13 ± 0.09	2.79 ± 0.13
Alkaline α-galactosidase	20 DAP	WW	1.73 ± 0.06^{a}	1.82 ± 0.06^{bc}	1.93 ± 0.06^{de}	2.15 ± 0.08
		WW + AMF	1.75 ± 0.07^{ab}	1.87 ± 0.05^{cd}	1.97 ± 0.05^{e}	2.23 ± 0.09
		DI	1.07 ± 0.04	1.28 ± 0.04	1.26 ± 0.04	1.54 ± 0.05
		DI + AMF	1.21 ± 0.05	1.74 ± 0.05	1.48 ± 0.07	2.11 ± 0.07
Alkaline α-galactosidase	30 DAP	WW	1.61 ± 0.07	1.72 ± 0.06	1.74 ± 0.06	1.81 ± 0.04
		WW + AMF	1.64 ± 0.05	1.77 ± 0.05	1.78 ± 0.04	1.87 ± 0.0
		DI	0.98 ± 0.03	1.19 ± 0.03	1.11 ± 0.03	1.27 ± 0.05
		DI + AMF	1.08 ± 0.05	1.64 ± 0.06	1.32 ± 0.03	1.76 ± 0.06

Note: [1]Here and in tables 3.10. and 3.11. the following conventions are used: RWC – relative water content; DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

However, the AMF did not significantly alter these parameters in the WW watermelon line, but significantly increased these parameters in the deficit irrigation in the complex and water deficit conditions, watermelon line. It may be that a sufficient amount of water relieves the difference between the mycorrhizal line and non-mycorrhizal line. In this study, the AMF effect on the RWC, PN and enzyme activities of the pumpkin-root line was relatively high compared to the corresponding own-root line.

Table 3.10. Fruit mass (kg) accumulation under different treatments during the fruit development stage (mean ± SE)

Stages	Treatments	2X	2X/P	3X	3X/P
10 DAP	WW	0.659 ± 0.028	0.741 ± 0.031	0.758 ± 0.032	0.801 ± 0.030
	WW + AMF	0.672 ± 0.031	0.762 ± 0.028	0.776 ± 0.029	0.829 ± 0.036
	DI	0.541 ± 0.024	0.652 ± 0.024	0.631 ± 0.027	0.708 ± 0.028
	DI + AMF	0.574 ± 0.023	0.712 ± 0.032	0.687 ± 0.023	0.786 ± 0.032
20 DAP	WW	2.808 ± 0.11	2.979 ± 0.11	3.021 ± 0.12	3.181 ± 0.14
	WW + AMF	2.859 ± 0.1	3.061 ± 0.13	3.085 ± 0.13	3.287 ± 0.12
	DI	2.262 ± 0.07	2.612 ± 0.09	2.529 ± 0.08	2.809 ± 0.09
	DI + AMF	2.411 ± 0.08	2.878 ± 0.12	2.778 ± 0.11	3.122 ± 0.12
30 DAP	WW	5.178 ± 0.21	5.669 ± 0.22	5.731 ± 0.21	6.168 ± 0.27
	WW + AMF	5.281 ± 0.22	5.841 ± 0.25	5.859 ± 0.19	6.371 ± 0.22
	DI	4.019 ± 0.13	4.779 ± 0.18	4.748 ± 0.20	5.261 ± 0.21
	DI + AMF	4.278 ± 0.16	5.412 ± 0.21	5.188 ± 0.23	6.032 ± 0.26

Table 3.11. The sucrose phosphate synthase (SPS) activity [μmol/g (glucose) h] under different treatments during the fruit development stage (mean ± SE)

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
SPS	10 DAP	WW	5.21 ± 0.18	4.76 ± 0.17	6.27 ± 0.25	5.51 ± 0.21
		WW + AMF	5.29 ± 0.21	4.93 ± 0.22	6.42 ± 0.28	5.75 ± 0.24
		DI	5.72 ± 0.23	5.68 ± 0.23	6.97 ± 0.31	6.85 ± 0.32
		DI+AMF	6.12 ± 0.26	6.51 ± 0.27	7.88 ± 0.34	7.96 ± 0.35
	20 DAP	WW	8.34 ± 0.27	7.61 ± 0.32	9.42 ± 0.42	8.64 ± 0.38
		WW + AMF	8.48 ± 0.36	7.84 ± 0.28	9.64 ± 0.37	8.96 ± 0.32
		DI	9.02 ± 0.35	8.75 ± 0.41	10.61 ± 0.48	10.12 ± 0.46
		DI + AMF	9.69 ± 0.41	9.93 ± 0.36	11.56 ± 0.52	11.81 ± 0.53
	30 DAP	WW	12.31 ± 0.42	11.39 ± 0.47	14.61 ± 0.62	13.89 ± 0.61
		WW + AMF	12.48 ± 0.55	11.71 ± 0.52	14.88 ± 0.58	14.38 ± 0.58
		DI	13.19 ± 0.41	12.78 ± 0.58	16.09 ± 0.71	15.81 ± 0.67
		DI + AMF	14.28 ± 0.35	14.89 ± 0.35	18.12 ± 0.42	18.72 ± 0.45

Although the AMF increased the RWC, PN and activities of the alkaline α-galactosidase, IAI, SuSy and SPS under both the DI and WW conditions, the effect was more obvious under the DI condition, which, as already noted, was accompanied by an increase in the concentration of salts in the soil solution.

AMF in the DI grafted plants increased the fruit yield to a level similar to the WW values. The sucrose and total sugar contents were highest in the DI + AMF treatment among all the treatments, and the sucrose and total sugar contents in the grafted line were higher than those in the corresponding own-root line. The fruit yield and total sugar content were highest in the pumpkin-root triploid line among all the

watermelon lines under the DI + AMF treatment. Integrated application of AMF and DI to pumpkin-root watermelon plants is a promising approach to enhance the fruit sugar content with negligible yield penalties, especially in a pumpkin-root triploid line.

So, the conducted studies proved that grafting and the use of arbuscular mycorrhizal fungi are factors against the background of which statistically reliable (at $p<0,05$) eco-physiological and biochemical changes occur in plants, which are mostly positive, including in terms of quality of the products received, and which contribute to increasing the resistance of plants to the influence of adverse environmental factors, including and salting.

Therefore, the research, the results of which are presented in the section, made it possible to form a comprehensive picture of the mechanisms of response and adaptation of pumpkin plants to salt stress and also reveal eco-physiological aspects of the formation of salt resistance, quantitative and qualitative characteristics of plants when using grafting technology and symbiotic interactions between plants and microorganisms. It is shown that the salt resistance of plants is determined by a complex of transformations that are implemented at different levels of the organization. The results prove not only the complexity of the problem of ensuring plant salt resistance, but also its great theoretical and practical importance and the possibility of its formation based on the use of both classical and modern technologies. Among the latter, approaches are promising, based on the use of interspecies symbiotic interactions are relationships.

The main research results presented in this section are covered in eight publications:

1. Yang P.M., **He S.T.**, Jiang L.N., Chen X.J., Li Y.F. & J.G. Zhou (2020). The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose content of different ploidy watermelon (*Citrullus lanatus*). Photosynthetica. 58 (5), 1150–1159. DOI: 10.32615/ps.2020.068
2. Xuejin Chena, **Songtao He**, Lina Jiang, Xinzheng Li, Weili Guo, Bihua Chena, Junguo Zhoua & Viktoriia Skliar (2021). An efficient transient transformation

system for gene function studies in pumpkin (*Cucurbita moschata* D.). Scientia Horticulturae, 1, 1–10. DOI:10.1016/j.scienta.2021.110028

3. Yang, P.M. & **He, S.T.** (2022). The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*). Hort. Sci. (Prague), 49б 225–233. https://doi.org/10.17221/108/2021-HORTSCI
4. **He Songtao**, Skliar V.G. & Zhou Junguo (2019). Effects of different concentrations of salt on pumpkin seedlings. Матеріали науково-практичної конференції викладачів, аспірантів та студентів Сумського НАУ (м. Суми, 17–20 квітня 2019 р.), 29.
5. **He Songtao**, Zhou Junguo & Skliar V.G. (2019). The problem of soil salinization and the role of genetic engineering in increasing the salt tolerance of plants. Матеріали Міжнародної науково-практичної конференції, присвяченої 90-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (м. Суми, 24–25 травня 2019 р.), 13.
6. **He Songtao**, Skliar V.G., Zhou Junguo & Xinxiang (2020). Effects of salt stress on the resistance of vegetable cytoplasmic membrane. Матеріали Міжнародної науково-практичної конференції, присвяченої 91-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича, (м. Суми, 25–26 травня 2020 р.), 44–45.
7. **He Songtao**, Zhou Junguo, Skliar V. H. & Xinxiang (2021). Mechanism of plant adaptation to osmotic stress. «Гончарівські читання»: Матеріали Міжнародної науково-практичної конференції, присвяченої 92-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (м. Суми, 25 травня 2021 р.), 108–109.
8. Хе Сунтао (2023). Вплив сольового стресу на розмір та віталітет рослин гарбуза. Матеріали науково-практичної конференції викладачів, аспірантів та студентів Сумського НАУ (м. Суми, 20–25 квітня 2023 р.), 57.

CONCLUSIONS

According to the results of the study of the influence of salt stress on pumpkin plants caused by different concentrations of NaCl (in the range of 0-120 mmol/L), as well as the mechanisms of formation of salt resistance at different levels of the organization, it was established:

1. The nature of the response of pumpkin morphological characters to salt stress was established. It is shown that against the background of increasing salt concentrations, there is a decrease in the size and vitality of plants and the manifestation of a number of negative quality signs in them: yellowing of leaves, their twisting, etc.

2. It is shown that plants respond to an increase in salt solution concentrations with a statistically significant increase the salt damage index and salt damage rate.

3. It has been proven that salt stress affects indicators and signs related to the course of photosynthesis and water exchange of pumpkin, leading to a decrease in the values of: photosynthetic rate, stomatal size, stomatal conductance, transpiration rate, as well as to changes in the content chlorophyll, mainly towards its increase. At the same time, a decrease in the indicators determining the amount of water loss are factors to increasing the resistance of plants to salt stress, and the indicators determining the intensity of photosynthesis are factors to slowing down the synthesis of carbohydrates and inhibiting plant growth.

4. An increase in the content of MDA and permeability of membrane structures of cells as a result of their peroxidation was registered in pumpkins under salt stress.

5. It is shown that transformations related to the exchange of carbohydrates and amino acids play an important role in the complex of processes of response and adaptation to salt stress in pumpkin: as salt concentrations increase, the content of proline and soluble sugars increases.

6. It was established that salt stress affects the absolute and relative indicators of the accumulation of ions of mineral substances, as well as their distribution by plant organs. According to these characteristics, Na^+, K^+, Ca^{2+} and Mg^{2+} ions demonstrated a high degree of individuality, which is also a result and evidence of their specific role both in the aspect of ensuring response and adaptation of pumpkin plants to salt stress.

7. It has been found out that in response and adaptation to salt stress in pumpkins at all levels of the organization against the background of the general trends, varietal features are clearly manifested. In particular, it was established that the Yanzhen mainly accumulates Na^+ in the root system, while the Miben mainly accumulates in the stem. This results in the formation of differences between varieties of the accumulation of K^+, Ca^{2+} and Mg^{2+}, as well as differences in the value of the K^+/Na^+ ratio. In general, according to a complex of physiological and morphometric characteristics, Yanzhen shows a higher resistance to salt.

8. Identification and analysis of the expression of the WRKY gene family in pumpkin under salt stress conditions was carried out. Screened 12 WRKY family transcription factors in pumpkin in response to salt stress, and analysed their phylogenetic relationships, spatio-temporal expression patterns, tissue-specific expression characteristics, and transcriptional activities under salt stress in detail.

9. For to improve and expand research aimed at revealing the genetic aspects of the formation of salt resistance, have developed an efficient transient transformation system for the study of gene function and protein subcellular localization. Using this method, the salt tolerance gene StNHX1 was transiently overexpressed in pumpkin roots and cotyledons.

10. The interactions that occur between the scion and the salt-resistant pumpkin rootstock when using the technology of grafting in the cultivation of gourds were studied. The influence of salt-resistant rootstocks on the metabolism of rootstocks has been clarified and proved, the feasibility of using grafting technology to increase the resistance of plants to salt stress, increase the yield of crops and obtain high-quality products has been shown.

11. It is shown that the use of arbuscular mycorrhizal fungi in the cultivation of melon crops based on grafting technology leads to a change in plant metabolism, affects the quality of the obtained products and contributes to increasing the resistance of plants to adverse environmental factors, including, to a certain extent, the increased salt content.

REFERENCES

1. Hossain, M. (2019). Present Scenario of Global Salt Affected Soils, its Management and Importance of Salinity Research. International Research Journal of Biological Sciences, 1, 1–3.

2. FAO. FAO Land and Plant Nutrition Management Service. Available online: http://www.fao.org/ag/ agl/agll/spush. 2008.

3. Zhou Heping, Zhang Lixin & Yu Feng (2007). Review and prospect of saline-alkali land improvement technology in China. Modern Agricultural Science and Technology, 11, 159-162 (in Chinese)

4. Zhang Jianfeng (2008). Principle and technology of ecological restoration of saline-alkali land. Beijing: China Forestry Publishing House , 14-15.

5. Liu Zuqi & Zhang Shicheng (1994). Plant Resistance Physiology. Beijing: China Agriculture Press, 222-223.

6. Rozema, J. & Flowers, T.J. (2008). Crops for a salinized world. Perspectives in Science, 322, 1478-1480.

7. Liang, W., Ma, X., Wan, P. & Liu. L. (2018). Plant salt-tolerance mechanism: A review. Biochemical and Biophysical Research Communications, 2018, 495, 286–291. doi: 10.1016/j.bbrc.2017.11.043

8. Flowers, T. J. & Colmer, T. D. (2008). Salinity tolerance in halophytes. New Phytol, 179 , 945–953. doi: 10.1111/j.1469-8137.2008.02531.x

9.Zhu, J. K. (2001). Plant salt tolerance. Trends in Plant Science, 6 (2), 66-71.

10. Xin Chen, Zongwen Zhang & Bin Wu. (2014). Comprehensive evaluation of salt tolerance of naked Oat at germination stage and screening of salt tolerant Germplasm. Chinese Journal of Agricultural Sciences, 47 (10), 2038-2046.

11. Chen Baoyue, Cao Ling & Wang Yanfang (2014). Effects of NaCl stress on growth, Physiological and biochemical characteristics and quality of celery. Journal of North China Agricultural Sciences, 218-222.

12. Wang Shuang, Li Xiaoxiao & Yu Chengzhi (2015). Effects of Salt Stress on growth and quality of three leaf Vegetables. Northern Horticulture, 20, 13-16. https://doi.org/10.3390/plants11212836

13. O'Leary, J.W. Adaptive ñomponents of salt tole rance (1995). Handbook of

plant and crop physiology. New York : Marcel Dekker Inc., 577–585.

14. Van Hoorn, J.W. & van Alphen, J.G. (2006). Salinity control Drainage Principles and Applications. – Wageningen, 533–600.

15. Ісаєнков, С.В. (2012). Фізіологічні та молекулярні аспекти сольового стресу рослин. Цитология и генетика, 46 (5), 50-71. doi: 10.3103/S0095452712050040

16. Деркач, В. & Романюк, Н. Д. (2016). Вплив засолення ґрунту на рослинні організми. Наук. зап. Терноп. нац. пед. ун-ту. Сер. Біол. 3-4. 67, 91-106.

17.Перерва, В. В. (2019).Аналіз солестійкості рослин морфометричним метод ом. Abstracts of I International Scientific and Practical Conference Osaka, Japan, 157-160.

18. Лебедева, А.Т. (1987). Тыквенные культуры. М: Россельхозиздат, 80.

19. Непочатов, О. П. Баштанні культури (1987). К. : Урожай, 1987, 176.

20. Caili, F., Huan, S. & Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods Hum. Nutr. 61, 73–80. DOI: 10.1007/s11130-006-0016-6

21. Yadav, M., Jain, S., Tomar, R., Prasad, G.B. & Yadav, H. (2010). Medicinal and biological potential of pumpkin: an updated review. Nutr. Res. Rev. 23, 184–190. DOI: 10.1017/S0954422410000107

22. Otani, T., Seike, N. & Sakata, Y. (2007). Differential uptake of dieldrin and endrin from soil by several plant families and Cucurbita genera. Soil Sci. Plant Nutr, 86–94. https://doi.org/10.1111/j.1747-0765.2007.00102.x

23. Лимар, О. А. Баштанництво України (2012). Миколаїв: МДАУ, 2012, 372.

24. Liu, H., Xu, J. W. & Wu, X. Q. (2001). Present situation and tendency of saline-alkalisoil in west Jilin Province. J. Journal of Geographical Sciences, 11 (3), 321–328.

25. Shi Yuanchun (1996). Saline-alkali soil improvement. Diagnosis, management and improvement. Beijing: Agriculture Press, 153

26. Zhu, J. K. (2001). Plant salt tolerance. Trends in Plant Science, 2001, 6 (2), 66–71. doi: 10.1016/S1360-1385(00)01838-0

27. Bian, J. M., Tang, J. & Lin, N. F. (2008). Relationship between saline-alkali soilformation and neotectonic movement in Songen Plain, China. Environ Geol, 55(7), 1421-1429

28. Fang, H. L., Liu, G. H. & Kearney, M. (2005). Georelational analysis of soil type, soil salt content, land form, and land use in the Yellow River Delta, China. Environmental Management, 2005, 35 (1), 72-83. doi: 10.1007/s00267-004-3066-2

29. Скляр, В.Г. (2015). Екологічна фізіологія рослин. Суми: Університетська книга, 271.

30. Amezketa, E. (2006). An integrated methodology for assessing soil salinization, a pre-condition for land desertification. Journal of Arid Environment, 67, 594–606. doi: 10.1016/j.jaridenv.2006.03.010

31. Huang, Mingyong, Zhang, Minsheng & Zhang, Xing (2009). Study on technical approaches of urban greening in coastal Saline-alkali Land Area. Review of 20 Years of Salt flat greening in Tianjin Development Area. Chinese Landscape Architecture, 9, 7.

32. Bartels, D. & Sunkar, R. (2005). Drought and salt tolerance in plants // Critical Reviews in Plant Sciences, 24, 1 : 23–58. doi: 10.1080/07352680590910410

33. Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. Annu Rev Plant Biol, 59? 651–681. doi: 10.1146/annurev.arplant.59.032607.092911.

34. Krasensky, J. & Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exper. Bot., 63, 4, 1593–1608. doi: 10.1093/jxb/err460

35. Horie, T, Karahara, I & Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. Rice, 5, 11. doi: 10.1186/1939-8433-5-11

36. Коваленко, Н.О. & Палладина, Т.О. (2016). Експресія генів Н(+)-насосів плазматичної та вакуолярної мембран клітин коренів кукурудзи за дії іонів натрію і біоактивних препаратів. Ukr. Biochem. J., 88, 2 : 89-97. doi: http://dx.doi.org/10.15407/ubj88.02.089

37. Munns, R. (2005). Genes and salt tolerance: bringing them together. New Phytol., 167, 645–663. doi: 10.1111/j.1469-8137.2005.01487.x

38. Zhu, J. K. Regulation ofion homeostasis under salt stress (2003). Curr Opin Plant Biol, 6, 441. doi: 10.1016/s1369-5266(03)00085-2

39. Василик, Ю.В. & Лущак, В.І. (2011). Вплив високих концентрацій хлориду натрію на вміст пігментів та вільнорадикальні процеси у листках проростків кукурудзи. Ukr. Biochem. J., 83, 4, 94-103

40. Jabeen, N. & Ahmad, R. (2012). Improvement in growth and leaf water relation parameters of sunflower and safflower plants with foliar application of nutrient solutions under salt stress. Pak. J. Bot , 44, 1341–1345.

41. Контурська, О.О. & Палладіна, Т.О. (2012). Активність ензимів аскорбат-глутамінового циклу в листках проростків кукурудзи в умовах засолення та абробки адаптогенними препаратами. Ukr. Biochem. J., 84, 6, 139-144.

42. Семчук, Н. М., Василик, Ю. В., Лущак, Ок. В. & Лущак В. І. (2012). Вплив короткотривалого сольового стресу на маркери оксидативного стресу та активність антиоксидантних ензимів у токоферол-дефіцитних рослин *Arabidopsis thaliana*. Ukr. Biochem. J., 84, 4, 41-48.

43. Muhammad, Akram, Shamshad, Akhtar & Ejaz, Rasul (2002). Impact of NaCl salinity on yield components of some wheat accessions/varieties. Int. J. Agr. Bio, 4, 156–158.

44. Haiying, Yu, Tingxuan, Li & Jianmin, Zhou (2005). Secondary Salinization of soil and its effects on soil properties. Soil, 37, 581–586.

45. Munns, R. Comparative physiology of salt and water stress (2002). Plant Cell Environ, 25, 239–250. doi: 10.1046/j.0016-8025.2001.00808.x

46. Zhu, J.K. (2001). Plant salt tolerance. Trends in Plant Science, 6 (2), 66–71. doi: 10.1016/S1360-1385(00)01838-0

47. Zhu, H. & Suyder, M. (2003). Protein chip technology. Current Opininon in Chemical Biology, 7 (1), 55–63. doi: 10.1016/S1367-5931(02)00005-4

48. Yamaguchi, T. & Blumwald, E. (2005). Developing salt-tolerant crop plants: challenges and opportunities. Trends Plant Sci., 10, 615–620. doi: 10.1016/j.tplants.2005.10.002

49. Sairam, R.K. & Srivastava, G. C. (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci, 162, 897–904. doi: 10.1016/S0168-9452(02)00037-7

50. Bose, J., Rodrigo-Moreno, A. & Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot, 65, 1241–1257. doi: 10.1093/jxb/ert430

51. Elkahoui, S., Smaoui, A., Zarrouk, M., Ghrir, R. & Limam, F. (2004). Salt-induced lipid changes in Catharanthus roseus cultured cell suspensions. Phytochemistry, 65, 1911–1917. doi: 10.1016/j.phytochem.2004.06.021

52. Chinnusamy, V., Jagendorf, A. & Zhu, J.K. (2005). Understanding and improving salt tolerance in plants. Crop Science, 45 (2), 437-448

53. Greenway, H. & Munns, R. (1980). Mechanisms of salt tolerance in non-halophytes. Annuals Review of Plant Physiology, 31, 149–190.

54. Zhu, J.K. (2003). Regulation of ion homeostasis under salt stress. Current Opininon in Plant Biology, 6 (5), 441–445. doi: 10.1016/S1369-5266(03)00085-2

55. Liu, J., Ishitani, M., Halfter, U., Kim, C.S. & Zhu, J.K. (2000). The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proceedings of the National Academy of Sciences of the United States of America, 97 (7), 3730–3734. doi: 10.1073/pnas.97.7.3730

56. Shabala, L., Cuin, T.A., Newman, I.A. & Shabala, S. (2005). Salinity-induced ion flux patterns from the excised roots of Arabidopsis SOS mutants. Planta, 222 (6), 1041–1050

57. Qiu, Q. S., Guo, Y., Dietrich, M. A., Schumaker, K.S. & Zhu, J.K. (2002). Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proceedings of the Nationgal Academy of Sciences of the United States of America, 99 (12), 8436–8441. doi: 10.1073/pnas.122224699

58. Cheng, N., Pittman, J.K., Zhu, J. & Hirschi, K. (2001). The protein kinase SOS2 activates the Arabidopsis H^+/Ca^{2+} antiporter CAX1 to integrate calcium

transport and salt tolerance. Journal of Biology Chemistry, 279 (4), 2922–2926. doi:10.1074/jbc.M309084200

59. Liu, J. & Zhu, J. K. (1998). A calcium sensor homolog required for plant salt tolerance. Science, 280 (5371), 1943–1945.

60. Liu, J., Ishitani, M., Halfter, U., Kim, C.S. & Zhu, J.K. (2000). The Arabidopsis thaliana SOS2 gene encodes a protein kinase that is required for salt tolerance. Proceedings of the National Academy of Sciences of the United States of America, 97 (7), 3730–3734. doi: 10.1073/pnas.97.7.3730

61. Shi, H., Ishitani, M., Kim, C. & Zhu, J.K. (2000). The Arabidopsis thaliana salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. Proceedings of the National Academy of Sciences of the United States of America, 2000, 97 (12), 6896–6901. doi:10.1073/pnas.120170197

62. Qiu, Q. S., Guo Y., Dietrich, M. A., Schumaker, K.S. & Zhu, J.K. (2002). Regulation of SOS1, a plasma membrane Na^+/H^+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3 Proceedings of the National Academy of Sciences of the United States of America, 99 (12), 8436–8441. doi:10.1073/pnas.122224699

63. Guo, Y., Halfter, U., Ishitani, M. & Zhu, J.K. (2001). Molecular characterization of functional domains in the protein kinase SOS2 that is required for plant salt tolerance. The Plant Cell, 13 (6), 1383–1399. doi:10.1105/tpc.13.6.1383

64. Shi, H. & Zhu, J.K. (2002). SOS4, a pyridoxal kinase gene, is required for root hair developmennt in Arabidopsis. Plant Physiology, 129 (2), 585–593. doi: 10.1104/pp.001982

66. Shi, H., Xiong, L., Stevenson, B., Lu, T. & Zhu, J.K. (2002). The Arabidopsis salt overly sensitive 4 mutants uncover a critical role for vitamin B6 in plant salt tolerance. Plant Cell, 14 (3), 575–588. doi: 10.1105/tpc.010417

67. Shi, H., Kim, Y.S., Guo, Y., Stevenson, B. & Zhu, J.K. (2003). The Arabidopsis SOS5 locus encodes a putative cell surface adhesion protein and is required for normal cell expansion. Plant Cell, 15 (1), 19–32. doi: 10.1105/tpc.007872

68. Mahajan, S., Pandey, G.K. & Tuteja, N. (2008). Calcium and salt-stress signaling in plants: Shedding light on SOS pathway. Archives of Biochemistry and Biophysics, 471 (2), 146–158. doi: 10.1016/j.abb.2008.01.010

69. Hsu, S.Y. & Kao, C.H. (2003). Differential effect of sorbitol and polyethylene glycol on antioxidant enzymes in rice leaves. Plant Growth Regulation, 39 (1), 83–90. doi: 10.1023/A:1021830926902

70. McCord, J.M. (2000). The evolution of free radicals and oxidative stress. The American Journal of Medicine, 108 (8), 652–659. doi: 10.1016/s0002-9343(00)00412-5

71. Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y. & Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. Journal of Experimental Botany, 53 (372), 1305–1319. doi: 10.1093/jexbot/53.372.1305

72. Tanaka, N., Mitsui, S., Nobori, H., Nobori, H. & Komatsu, S. (2005). Expression and function of proteins during development of the basal region in rice seedlings. Molecular and Cell Proteomics, 4 (6), 796–808. doi: 10.1074/mcp.M400211-MCP200

73. Gueta-Dahan, Y., Yaniy, Z., Zilinskas, B.A. & Ben-Hayyim, G. (1997). Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. Planta, 203 (4), 460–469. doi: 10.1007/s004250050215

74. Meneguzzo, S., Navari-Izzo, F. & Izzo, R. (1999). Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. Journal of Plant Physiology, 155 (2), 274–280. doi: 10.1016/S0176-1617(99)80019-4

75. Bayuelo-Jiménez, J.S, Debouck, D.G. & Lynch, J.P. (2003). Growth, gas exchange, water relations, and ion composition of Phaseolus species grown under saline conditions. Field Crops Research, 80 (3), 207–222.

76. Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. & Thomas, G. (2003). Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) – differential response in salt-tolerant and sensitive varieties. Plant Science, 165 (6), 1411–1418. doi: 10.1016/j.plantsci.2003.08.005

77. Yazici, I., Türkan, I., Sekmen, A.H. & Demiral, T. (2007). Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environmental and Experimental Botany, 61 (1), 49–57. doi: 10.1016/j.envexpbot.2007.02.010

78. Wang, J., Zhang, H. & Allen, R.D. (1999). Over expression of an Arabidopsis peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. Plant and Cell Physiology, 40 (7), 725–732.

79. Roxas, V.P., Lodhi, S.A., Garrett, D.K., Mahan, J.R. & Allen, R.D. (2000). Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant and Cell Physiology, 41 (11), 1229–1234 doi: 10.1093/pcp/pcd051

80. Badawi, G.H. Kawano, N., Yamauchi, Y., Shimada, E., Sasaki, R., Kubo, A. & Tanaka, K. (2004). Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiologia Plantarum, 121 (2), 231–238. doi: 10.1111/j.0031-9317.2004.00308.x

81. Jie Chen & Xifeng Lin (2003). Research progress on physiology and mechanism of salt tolerance in plants. Journal of Hainan University (Natural Science Edition), 21 (2), 177–182

82. Smirnoff, N. & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28 (4), 1057–1060. doi: 10.1016/0031-9422(89)80182-7

83. Liu, J. P. & Zhu, J. K. (1997). Proline accumulation and salt-stress-induced gene expression in salt-hypersensitive mutant of Arabidopsis. Plant Phssiology, 114 (2), 591–596. doi: 10.1104/pp.114.2.591

84. Діденко, Н. О., Волков, Р. А. & Панчук, І. І. (2016). Вплив сольового стресу на вміст проліну та поліфенольних сполук у *Arabidopsis thaliana*. Біологічні системи, 8 (1) : 35-39

85. Yoshiba, Y., Kiyosue, T., Kataggiri, T., Ueda, H., Mizoguchi, T., Yamaguchi-Shinozaki, K., Wada, K., Harada, Y. & Shinozaki, K. (1995). Correlation between the induction of a gene for A-pyrroline-5-carboxylate synthetase and accumulation

of proline in *Arabidopsis thaliana* under osmotic stress. Plant Journal, 7 (5), 751–760. doi: 10.1046/j.1365-313x.1995.07050751.x

86. Zhao Kefu (1995). Halophytes of China. International Symposium on High Salinity Tolerant Plants Volume: Biology of Salt Tolerant Plants, 284–293.

87. Babivchuk, E., Kushnir, S., Belles-Boix, E., Montagu, M. & Inzé, D. (1995). *Arabidopsis thaliana* NADPH oxidoreductase homologs confer tolerance of yeasts towards the thiol-oxidizing drug diamide. Journal of Biological Chemistry, 270 (44) : 26224–26231. doi: 10.1074/jbc.270.44.26224

88. Demiral, T. & Türkan, I. (2006). Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environmental and Experimental Botany, 56 (1), 72–79. doi: 10.1016/j.envexpbot.2005.01.005

89. Park, E.J., Jeknic, Z. & Chen, T.H. (2006). Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. Plant and Cell Physiology, 47 (6), 706–714. doi: 10.1093/pcp/pcj041

90. Ma, Q.Q., Wang, W., Li, Y.H., Li, D.Q. & Zou, Q. (2006). Alleviation of photoinhibition in drought-stressed wheat (Triticum aestivum) by foliar-applied glycinebetaine. Journal of Plant Physiology, 163 (2), 165–175. doi: 10.1016/j.jplph.2005.04.023

91. Raza, S.H., Athar, H.R., Ashraf, M. & Hameed, A. (2007). Glycine betaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environmental and Experimental Botany, 60 (3), 368–376

92. Hoque, M.A., Banu, M.N.A., Okuma, E., Amako, K., Nakamura, Y., Shimoishi, Y. & Murata, Y. (2007). Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. Journal of Plant Physiology, 164 (11), 1457–1468. doi: 10.1016/j.jplph.2006.10.004

93. Tamura, T., Hara, K., Yamaguchi, Y., Koizumi, N. & Sano, H. (2003). Osmotic stress tolerance of transgenic tobacco expressing a gene encoding a membrane-

located receptor-like protein from tobacco plants. Plant Physiology, 131 (2), 454–462. doi: 10.1104/pp.102.011007

94. Zhang Xiaolei, Nie Yuzhe & Li Yuhua (2008). Cell signal transduction in plants under salt stress. Biotechnology Communications, 19 (3), 468–471.

95. Barkla, B.J. & Pantoja, O. (1996). Physiology of ion transport across the tonoplast ofhigher plants. Annual Review of Plant Physiologyand Plant Molecular Biology, 47, 159–184. doi: 10.1146/annurev.arplant.47.1.159

96. Liu Yan, Peng Xuexian, Xie Youju & Dai Jingrui (1997). Advances in genetic engineering of plant resistance to osmotic stress. Advances in Bioengineering, 17 (2), 31–38.

97. Chen Guanping, Wang Huizhong, Shi Nongnong & Chen Shouyi (2006). Advances in the relationship between Na+/H+ reverse transporters and salt tolerance in plants. Chinese Journal of Biological Engineering, 26 (5), 101–106.

98. Коваленко, Н.О., Білик, Ж.І. & Палладіна, Т.О. (2014). Вплив адаптогенних препаратів на функціонування $Na(^{+})/H(^{+})$-антипортера плазматичної мембрани в клітинах коренів кукурудзи за умов засоленого середовища. Ukr. Biochem. J., 86, 5, 134-141

99. Yamada, S., Katsuhara, M. & Kelly, W. B. (1995). A family of transcripts encoding water channel proteins: tissue-specific expression in the common ice plant. Plant Cell, 7, 1129–1142. doi: 10.1105/tpc.7.8.1129

100. Maurel, C. (1997). Aquaporins and water permeability of plant membranes. Annual Review Plant Physiology and Plant Molecular Biology, 48, 399–429. doi: 10.1146/annurev.arplant.48.1.399

101. Suga, S., Komatsu, S. & Maeshima, M. (2002). Aquaporin isoforms responsive to salt and water stresses and phytohormones in radish seedlings. Plant and Cell Physiology, 43(10), 1229–1237. doi: 10.1093/pcp/pcf148

102. Висилик, Ю.В., Семчук, Н.М., Лущак, Ок.В. & Лущак, В.І. (2012). Вплив нітропрусиду та хлориду натрію на вміст карбонільних груп протеїнів та активність антиоксидантних ензимів у листках проростків кукурудзи *Zea mays* L. Ukr. Biochem. J., 84, 3, 82-87

103. Ястреб, Т.О., Колупаєв, Ю.Є., Швиденко, М.В. & Дмитрієв, О.П. (2018). Дія метилжасмонату і сольового стресу на антиоксидантну систему рослин арабідопсису, дефектних за генами жасмонатного сигналінгу. Ukr. Biochem. J., 90, 5, 50-59. doi: https://doi.org/10.15407/ubj90.05.050

104. Ingram, J. & Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 47, 377–403. doi: 10.1146/annurev.arplant.47.1.377

105. Wu, S. J, Ding, L. & Zhu, J. K. (1996). SOS1, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell, 8 (4), 617–627. doi: 10.1105/tpc.8.4.617

106. Pandey, A. & Mann, M. (2000). Proteomics to study genes and genomes. Nature, 405 (6788), 837–846. doi: 10.1038/35015709

107. Tanaka, N. & Mitsui, S. (2005). Expression and function of proteins during development of the basal regior in rice seedlings. Molecular and Cell Proteomics, 4(6), 796–808. doi: 10.1074/mcp.M400211-MCP200

108. Кощій, О.В. Проблеми забезпечення населення України продовольством (2013). Соц.-ек.проблеми сучас.періоду України. Вип. 6 (104) 4, 441-448.

109. Сидора, В.В. (2017). Формування та розвиток маркетингу на ринку овочевої продукції. Економіка та управління підприємствами, 4 (60), 111-118.

110. Писаренко, В.В. (2010). Перспективи розвитку галузі оочівництва. Економіка АПК. 7, 39–42.

111. Сухий, П.А. & Заячук, М.Д. (2012). Сучасний стан і перспективи розвитку овочівництва в Україні. Вчені записки Таврійського національного університету ім. В.І. Вернадського. Серія: Географічні науки. 25 (64), 3, 38–48.

112. Корнієнко, С.І., Горова, Т.К. & Рудь, В.П. (2016). Галузева програма «Овочі України – 2020». Х.: «Плеяда», 65.

113. Корнієнко, С.І., Рудь, В.П., Кіях, О.О. & Терьохіна Л.А. (2012). Концептуальні основи розвитку овочівництва та забезпечення продовольчої безпеки. Міжвідомчий тематичний науковий збірник «Овочівництво і баштанництво», 58, 7–18.

114. Сировицький, К.Г. & Харченко, С.О. (2019). Матеріали МНПК «Інноваційні розробки в аграрній сфері», ХНТУСГ, ННІ МСМ, 12-13 грудня 2019 року, 191.

115. Онипко, В.В. & Таран, І.О. (2009). Особливості росту та розвитку перспективних сортів виду *Cucumis mello* L. Проблеми відтворення та охорони біорізноманіття України в світлі вчення про ноосферу. Матеріали Всеукраїнської студентської науково-практичної конференції. Полтава: Астрая, 119-121.

116. Лендел, В.Ф. (2014). Особливості росту і розвитку рослин та урожайність гарбуза мускатного залежно від віку розсади за розсадного способу вирощування. Агробіологія, 2014. № 1 (109), 81-84.

117. Хареба, В.В. & Кокойко, В.В. (2015). Використання природних регуляторів росту рослин (РРР) у технологіях вирощування гарбуза мускатного (*Cucurbita moschata* Duch. ex Poir.). Овочівництво і баштанництво, (61), 320-326117.

118. Гончаренко, В.Ю., Парамонова, Т.В., Могильна, О.М., Міхайлін, В.І., & Мозговский, О. Ф. (2019). Система удобрення овочевих та баштанних культур. К.: Аграрн. Наука, 152

119. Вдовенко, С.А. & Паламарчук, І.І. (2021). Інновації в технології вирощування овочевих рослин родини Гарбузові у відкритому ґрунті. Вінниця, 184.

120. Лимар, А., & Холодняк, О. (2021). Ефективність використання стимуляторів росту при вирощуванні кавуна столового в умовах півдня України. Овочівництво і баштанництво, (69), 99-109. https://doi.org/10.32717/0131-0062-2021-69-99-109

121. Сич, З. Д., Колесник, І. І., Діденко, В. П. та ін. (2001). Кавун, диня, гарбуз. Сучасні методи селекції овочевих і баштанних культур, 644 .

122. Колесник, І.І. (2014). Генетичні ресурси гарбуза великоплідного в селекції на насіннєву продуктивність. Овочівництво і баштанництво. 60, 128-136.

123. Колесник, І.І. (2014). Спосіб селекції гарбуза на скоростиглість. Овочівництво і баштанництво, 60, 124-127.

124. Ліннік, З.П., Чаюк, О.О., Сергієнко, О.В. & Онищенко, О.І. (2021). Вихідний матеріал кавуна для селекції на комплексну стійкість до хвороб. Овочівництво і баштанництво, (69), 13-23. https://doi.org/10.32717/0131-0062-2021-69-13-23

125. Сергієнко, О. & Лінник, З. (2022). Рівень зв'язку між ознаками колекційних сортозразків кавуна. Овочівництво і баштанництво, (71), 16-24. https://doi.org/10.32717/0131-0062-2022-71-16-24

126. Кондратенко, С. & Ланкастер, Ю. (2022). Важливі кореляційні взаємозалежності між комплексом господарськоцінних ознак гібридів F1 кабачка в аспекті їх адаптивного потенціалу. Овочівництво і баштанництво, (71), 6-15. https://doi.org/10.32717/0131-0062-2022-71-6-15

127. Сергієнко, О. & Лінник З. (2023). Адаптивний потенціал колекції гібридів F1 кавуна за продуктивними показниками. Овочівництво і баштанництво, (72), 32-40. https://doi.org/10.32717/0131-0062-2022-72-32-40
14.

128. Сергієнко, О., Шабетія, О., Івченко, Т., Гарбовська, Т., Солодовник, Л. & Радченко, Л. (2022). Оцінка нових партенокарпічних гібридних комбінацій f1 огірка за цінними селекційними ознаками та їх мінливістю в умовах захищеного грунту. Овочівництво і баштанництво, (71), 25-32. https://doi.org/10.32717/0131-0062-2022-71-25-32

129. Бобось, І. М., & Лаврентьєва, Н. О. (2013). Інтродукція малопоширених овочевих культур родини Гарбузові. Plant Varieties Studying and Protection, 1 (18), 47–50. https://doi.org/10.21498/2518-1017.1(18).2013.58751

130. Гуцол, Н. М. & Журавель, Н. М. (2018). Екзотичні рослини родини *Cucurbitaceae*, що культивуються в Україні. Матеріали III Міжнародної науково-практичної конференції «Сучасний рух науки», 1-2 жовтня 2018 р., 155-159

131. Georg, R. (1980). Horticultura in Hungaru. Sci. Hort. 31, 23-27.

132. Loy, J. B. (1982). Autumn Pride winter Squash. Hort. Science. 17 (5), 832-833.

133. Соколов, Д. Ю. (1996). Тыква – семенная продуктивность, выход масла и его жиронокислотный состав. Материалы международной научной конференции, 150-152

134. Колесник, І. І. & Сич, З. Д. (1996). Багатонасінний гарбуз – перспективна олійна культура для України. Матеріали міжнародної наукової конференції, 44-46.

135. FAO Production Yerbook (2002). Roome, 55, 416

136. Ferriol M. & Pico B. (2008). Pumpkin and winter squash. In: Vegetables I (edited by J. Prohens & F. Nuez). New York: Springer., 317- 349.

137. Wu, P.C. (1994). The Biology and Cultivation Techniques of Melons. Beijing: China Agriculture Press, 114-122.

138. Лымар, В. А., Григоров, Ю. Г. & Лымар, А. О. (2011). Бахчевые культуры в лечебно-профилактическом питании. Херсон: Айлант, 252

139. Колесник, І. І. (2015). Джерела господарсько-цінних ознак культурних видів гарбуза для різних напрямів селекції. Наукові доповіді НУБІП України. [Електронний ресурс]. – Режим доступу: http://nbuv.gov.ua/UJRN/Nd_2015_4_20

140. Shu Yingchun (1998). Brief history of cultivation of main melon vegetables. Agricultural History of China, 17 (3), 94-99

141. Lindepei (2000). Origin and classification of the pumpkin plant. Chinese Watermelon and Melon, 2000 (1), 36-38

142. Lu Bin (1982). Physiology of Vegetables and Melons. Beijing : Agriculture Press, 376-379

143. Yang Xiuling, Yu Jihua & Li Yajia (2004). Effects of NaCl stress on seed germination and seedling growth of cucumber. Journal of Gansu Agricultural University, 39 (1), 6-9

144. Wang Ran, Cai Run & Pan Junsong (2005a). Effects of salt stress on germination characteristics of cucumber seeds, Journal of Shanghai Jiao Tong University (Agricultural Science Edition), 23 (2), 148-153

145. Wang Ran, Chen Guilin & Liang Jinget (2005 b). Effects of salt stress on seed germination characteristics of black-seeded pumpkin and white-seeded pumpkin. Journal of Agricultural University of Hebei. 28 (5), 42-44

146. Wang Guangyin, Han Shidong & Zhao Yipenget (2005). Effects of NaCl stress and Ca^{2+} and GA3 on seed germination of three vegetable species of Pumpkin. Journal of Plant Resources and Environment, 14 (1), 26-30

147. Li Weixin, Chen Guilin & Zhao Li (2006). Salt tolerance of different pumpkin seedlings under NaCl stress. Journal of Plant Genetic Resources, (2), 192-196.

148. Zhu Jin, Beizhi Dragon & Li Yana (2006). Evaluation of salt tolerance of cucumber seed at germination stage and grafting rootstock at seedling stage. Scientia Agricultura Sinica, 39 (4), 772-778

149.Wang Guangyin, Zhou Xiumei & Zhang Jianweiet (2005 b). Effects of NaC1 stress on germination of cucumber seeds. Agricultural Research in the Arid Areas, 23 (1), 121-125

150. Tang Juxiang, Li Xinzheng & Li Guangling (2005). Study on salt tolerance of different varieties of pumpkin seedlings. Journal of Anhui Agricultural Sciences, 33 (10), 1802-1839

151. Wang Ran, Chen Guilin & Song Wei (2006). Effects of NaCl stress on ion content in two kinds of pumpkin seedlings. Chinese Journal of Plant Physiology and Molecular Biology, 32 (1), 94-98

152. Wang Suping, Li Juan & Guo Shirong (2006). Effects of NaCl stress on growth and photosynthetic characteristics of cucumber seedlings. Acta Botanica Sinica, 26 (3), 455-461

153. Sivritepe, N., Sivritepe, H. O. & Eris, A. (2003). The effects of NaCl priming on salt tolerance in melon seedlings grown under saline condition. Scientia Horticulture, 97, 229-237. https://doi.org/10.1016/S0304-4238(02)00198-X

154. Sivritepe, H. O., Sivritepe, N. & Eris, A. (2005). The effects of NaC1 pre-treatments on salt tolerance of melons grown under long-term salinity. Scientia Horticulturae, 106, 568-581

155. Zhang Yuxin, Li Xie & Chen Nianlai (2006). Effects of NaCl stress on the growth of melon seedlings. Chinese Melons and Vegetables, (10), 4-6

156. Suping Wang, Shirong Guo, Jing Li, Xiaohui Hu & Yansheng Jiao (2006). Effects of salt stress on photosynthetic pigment content in cucumber seedling leaves. Journal of Jiangxi Agricultural University, 28 (1), 32-38

157. Zhang Enping, Zhang Shuhong, Si Longting, Pang Jinan & Ma Dehua (2001). Effects of NaCl stress on membrane lipid peroxidation in cotyledon of cucumber seedlings. Journal of Shenyang Agricultural University, 32 (6), 446-448

158. Wei Guoqiang, Zhu Zhujun & Fang Xuezhi (2004). Effects of NaCl stress on growth, chlorophyll fluorescence characteristics and active oxygen metabolism of cucumber seedlings. Scientia Agricultura Sinica, 37 (11), 1754-1759

159. Villora, G., Moreno, D. A., Pulgar, G., & Romero, L. (2000). Yield improvement in zucchini under salt stress: determining micronutrient balance. Scientia Horticulturae, 2000, 86, 175-183 DOI:10.1016/S0304-4238(00)00149-7

160. Sun, H., Wu, S., Zhang, G., Jiao, C., Guo, S., Ren, Y., Zhang, J., Zhang, H., Gong, G., Jia, Z., Zhang, F., Tian, J., Lucas, W.J., Doyle, J.J., Li, H., Fei, Z. & Xu, Y. (2017). Karyotype stability and unbiased fractionation in the Paleo-allotetraploid Cucurbita genomes. Mol. Plant 10, 1293–1306. https://doi.org/10.1016/j.molp.2017.09.003

161. Xue, G.P. (2003). The DNA-binding activity of an Ap2 transcriptional activator Hvcbf2 involved in regulation of low-temperature responsive genes in barley is modulated by temperature. Plant J, 33 (2), 373-383. DOI: 10.1046/j.1365-313x.2003.01630.x

162. Riechmann, J.L., Heard, J. & Martin, G. (2000). Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science, 290 (5499), 2105-2110. DOI: 10.1126/science.290.5499.2105

163. Yamasaki, K., Kigawa, T. & Inoue M. (2008). Structures and evolutionary origins of plant-specific transcription factor DNA-binding domains. Plant Physiology and Biochemistry, 4(3), 394-401. DOI: 10.1016/j.plaphy.2007.12.015

164. Ishiguro, S. & Nakamura, K. Characterization of a cDNA encoding a novel DNA-binding protein, Spf1, that recognizes Sp8 sequences in the 5’upstream

regions of genes coding for sporamin and beta-amylase from sweet potato (1994). Molecular and General Genetics, 244 (6), 563-571. DOI: 10.1007/BF00282746

165. Rushton, P.J., Macdonald, H. & Huttly, A.K. (1995). Members of a new family of DNA-binding proteins bind to a conserved Cis-element in the promoters of A-Amy2 genes. Plant Mole Biol, 29 (4), 691-702. DOI: 10.1007/BF00041160

166. Eulgem, T., Rushton, P.J. & Robatzek, S. (2005). The WRKY superfamily of plant transcription factors. Trends in Plant Science, 5(5), 199-206. DOI: 10.1016/s1360-1385(00)01600-9

167. Rushton, P.J., Somssich, I.E., Ringler, P. & Shen, Q.J. (2010) WRKY transcription factors. Trends in Plant Science, 15 (5), 247-258. DOI: 10.1016/j.tplants.2010.02.006

168. Maeo, K, Hayashi, S, Kojima, S.H, Morikam, A. & Nakamura, K. (2001). Role of conserved residues of the WRKY domain in the DNA-binding of tobacco WRKY family proteins. Bioscience Biotechnology, 65 (11), 2428-2436. DOI: 10.1271/bbb.65.2428

169. Ülker, B. & Somssich, I.E. (2004). WRKY transcription factors: from DNA binding towards biological function. Current Opinion in Plant Biology, 7 (5), 491-498. DOI: 10.1016/j.pbi.2004.07.012

170.Bakshi, M. & Oelmüller, R. (2014). WRKY transcription factors ：Jack of many trades in plants. Plant Signaling Behavior, 9 (2). DOI: 10.4161/psb.27700

171.Yamasaki, K, Kigawa, T. & Inoue, M. (2005). Solution structure of an Arabidopsis WRKY DNA binding domain. The Plant Cell, 17 (3), 944-956. DOI: 10.1105/tpc.104.026435

172. Zhang, Y. & Wang, L. (2005). The WRKY transcription factor superfamily: its origin in eukaryotes and expansion in plants. BMC Evol Biol, 5, 1 https://doi.org/10.1186/1471-2148-5-1

173. Wan, Y., Mao, M. & Wan, D. (2018). Identification of the WRKY gene family and functional analysis of two genes in Caragana intermedia. BMC Plant Biol, 18, 31. https://doi.org/10.1186/s12870-018-1235-3

174. Chen, L., Song, Y. & Li, S. (2012). The role of WRKY transcription factors in plant abiotic stresse. Biochimica et Biophysica Acta-Gene Regulatory

Mechanisms, 1819 (2), 120-128. DOI: 10.1016/j.bbagrm.2011.09.002

175. Park, C.Y, Lee, J.H. & Yoo, J.H. (2005). WRKY group iid transcription factors interact with calmodulin. FEBS Letters, 579 (6), 1545-1550. DOI: 10.1016/j.febslet.2005.01.057

176. Meng, D., Li, Y. & Bai, Y. (2016). Genome-wide identification and characterization of WRKY transcriptional factor family in apple and analysis of their responses to waterlogging and drought stress. Plant Physiology, 103, 71-83. https://doi.org/10.1016/j.plaphy.2016.02.006

177. Tamura, K., Dudley, J. & Nei, M. (2007). Mega4: Molecular evolutionary genetics analysis（MEGA）software version 4. 0. Molecular Biology Evolution, 24 (8), 1596-1599. DOI: 10.1093/molbev/msm092

178. Ou, C., Jiang, S. & Wang, F. (2015). An RNA-Seq analysis of the pear（*Pyrus communis* L.）transcriptome, with a focus on genes associated with dwarf. Plant Gene, 4, 69-77. https://doi.org/10.1016/j.plgene.2015.08.003

179. Zhang, D., Ren, L. &Yue, J.H. (2015). RNA-Seq-based transcriptome analysis of stem development and dwarfing regulation in Agapanthus praecox ssp. Orientalis (Leigh) Leighton. Gene, 565(2), 252-267. DOI: 10.1016/j.gene.2015.04.013

180. Jiang, J., Ma, S. & Ye, N. (2017). WRKY transcription factors in plant responses to stresses. Journal of Integrative Plant Biology, 59 (2), 86-101. DOI: 10.1111/jipb.12513

181. Cai, Y., Chen, X. & Xie, K. (2014). Dlf1, a WRKY transcription factor, is involved in the control of flowering time and plant height in rice. PLoS One, 9（7). DOI: 10.1371/journal.pone.0102529

182. Guo, D., Zhang, J. & Wang, X. (2015). The WRKY transcription factor WRKY71/EXB1 controls shoot branching by transcriptionally regulating RAX genes in Arabidopsis. The Plant Cell, 27 (11), 3112-3127. https://doi.org/10.1105/tpc.15.00829

183. Ma, Y., Xue, H. & Zhang, L. (2016). Involvement of auxin and brassinosteroid in dwarfism of autotetraploid apple (Malus×domestica). Scientific Reports, 6, 26719

184. Soumelidou, K., Morris, D. & Battey, N. (1994). Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. Journal of Horticultural Science, 69 (4), 719-725. https://doi.org/10.1080/14620316.1994.11516505

185. Michalczuk, L.J. (2002). Indole-3-acetic acid level in wood, bark and cambial sap of apple rootstocks differing in growth vigour. Acta Physiologiae Plantarum, 24 (2), 131-136

186. Zhao, B. & Li, J.J. (2012). Regulation of brassinosteroid biosynthesis and inactivation. Journal of Integrative Plant Biology, 54 (10), 746-759. https://doi.org/10.1111/j.1744-7909.2012.01168.x

187. Zheng X., Zhao Y., Shan D., et al. Mdwrky9 overexpression confers intensive dwarfing in the M26 rootstock of apple by directly inhibiting brassinosteroid synthetase Mddwf4 expression. New Phytologist, 2018, 217 (3), 1086-1098. DOI: 10.1111/nph.14891

181. Xie, Z., Zhang, Z.L. & Zou, X. (2005). Annotations and functional analyses of the rice WRKY gene superfamily reveal positive and negative regulators of abscisic acid signaling in aleurone cells. Plant Physiology, 137 (1), 176-189. DOI: 10.1104/pp.104.054312

182.Qiu, D., Xiao, J. & Ding, X. (2007). OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate-and jasmonate-dependent signaling. Molecular Plant-Microbe Interactions, 20 (5), 492-499. DOI: 10.1094/MPMI-20-5-0492

183. Zhang, J., Peng, Y. & Guo, Z.J. (2008). Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. Cell Research, 18 (4), 508.

184. Wang, P.T., Xu, X. & Tang Z. (2018). OsWRKY28 regulates phosphate and arsenate accumulation, root system architecture and fertility in rice. Frontiers in Plant Science, 9, 1330.

185. Hu, Z., Wang, R. & Zheng, M. (2018). Ta WRKY 51 promotes lateral root formation through negative regulation of ethylene biosynthesis in wheat（Triticum aestivum L.. Plant J, 96（2）：372- 388. DOI: 10.1111/tpj.14038

186. Cheng, Y., Ahammed, G.J. & Yu, J. (2016). Putative WRKYs associated with

regulation of fruit ripening revealed by detailed expression analysis of the WRKY gene family in pepper. Scientific Reports, 6. doi ：10.1038/srep39000.

187.Yang, X., Li, H. & Yang, Y. (2018). Identification and expression analyses of WRKY genes reveal their involvement in growth and abiotic stress response in watermelon（*Citrullus lanatus*). PLoS One, 13 (1).

188. Li, Q.L., Bo, S. & Deng, W.J. (2019). Avocado fruit pulp transcriptomes in the after-ripening process. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47 (2), 308-319. DOI: https://doi.org/10.15835/nbha47111346

189. Li, D., Mou, W. & Xia. R. (2019). Integrated analysis of high-throughput sequencing data shows abscisic acid-responsive genes and mirnas in strawberry receptacle fruit ripening. Horticulture Research, 6 (1), 26.

190. Guo, Y., Cai, Z. & Gan, S. (2004). Transcriptome of Arabidopsis Leaf senescence. Plant, Cell Environment, 27 (5), 521-549. https://doi.org/10.1111/j.1365-3040.2003.01158.x

191. Robatzek, S. & Somssich, I.E.J. (2001). A new member of the Arabidopsis WRKY transcription factor family, AtWRKY6, is associated with both senescence-and defence-related processes. Plant J., 28 (2), 123-133. DOI: 10.1046/j.1365-313x.2001.01131.x

192. Robatzek, S. & Somssich, I.E.J. (2002). Targets of AtWRKY6 regulation during plant senescence and pathogen defense. Gene Development, 16 (9), 1139-1149. DOI: 10.1101/gad.222702

193. Gu, L. &Wei, H. (2018). Characterization and functional analysis of Ghwrky42, a group IId WRKY gene, in upland cotton（*Gossypium hirsutum* L.). BMC Genetics, 19 (1), 48.

194. Fan, Z.Q., Tan, X.L. & Shan, W. (2018). Characterization of a transcriptional regulator, BrWRKY6, associated with gibberellin-suppressed leaf senescence of chinese flowering cabbage. Journal of Agricultural Food Chemistry, 66 (8), 1791-1799. DOI: 10.1021/acs.jafc.7b06085

195. Jones, J.D.G., & Dangl, J.L. (2006). The plant immune system. Nature, 444 (7117), 323-329.

196. Monaghan, J. & Zipfel, C. (2012). Plant pattern recognition receptor

complexes at the plasma membrane. Current Opinion in Plant Biology, 15 (4), 349-357. DOI: 10.1016/j.pbi.2012.05.006

197. Chisholm, S.T., Coaker, G. & Day, B. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. Cell, 124 (4), 803-814. https://doi.org/10.1016/j.cell.2006.02.008

198. Ifnan, K.M., Zhang, Y. & Liu, Z. (2018). Cawrky40b in pepper acts as a negative regulator in response to Ralstonia solanacearum by directly modulating defense genes including CaWRKY40. International journal of Molecular Sciences, 19 (5), 1403. doi: 10.3390/ijms19051403

199. Wang, Z., Zhu, Y. & Wang, L. (2009). A WRKY transcription factor participates in dehydration tolerance in Boea hygrometrica by binding to the W-Box elements of the galactinol synthase (BhGOLS) promoter. Planta, 230 (6), 1155-1166. doi: 10.1007/s00425-009-1014-3

200. Hussain, R.M., Sheikh, A.H. & Haider, I. (2018). Arabidopsis WRKY50 and TGA transcription factors synergistically activate expression of Pr1. Front Plant Sci, 9, 930. https://doi.org/10.3389/fpls.2018.00930

201. Miao, Y. & Laun, T.M. (2007). Arabidopsis MEKK1 can take a short cut ： It can directly interact with senescence-related WRKY53 transcription factor on the protein level and can bind to its promoter. Plant Mole Biol, 65（1-/2), 63-76. DOI: 10.1007/s11103-007-9198-z

202. Sheikh, A.H. & Eschen, L.L. (2016). Regulation of WRKY46 transcription factor function by mitogen-activated protein kinases in Arabidopsis thaliana. Front Plant Sci, 7, 61. DOI: 10.3389/fpls.2016.00061

203. Pandey, S.P. & Somssich, I.E.J. (2009). The role of WRKY transcription factors in plant immunity. Plant Physiology, 150 (4), 1648-1655. DOI: 10.1104/pp.109.138990

204. Arraño, S.P, Domínguez, F.J. & Herrera, V.A. (2018). WRKY7, -11 and- 17 transcription factors are modulators of the Bzip28 branch of the unfolded protein response during pamp-triggered immunity in Arabidopsis thaliana. Plant Science, 277, 242-250.

205. Çelik, Ö., Meriç. S. & Ayan. A. (2019). Epigenetic analysis of WRKY

transcription factor genes in salt stressed rice （Oryza sativa L.） plants. Environmental Experimental Botany, 159 , 121-131.

206. Pandey, N. & Pandey, R.S.J. Deciphering UV-B-induced variation in DNA methylation pattern and its influence on regulation of DBR2 expression in Artemisia annua L. Planta, 2015, 242 (4), 869-879. DOI: 10.1007/s00425-015-2323-3

207. Kim, K.C., Lai, Z. & Fan, B. (2008). Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. The Plant Cell, 20 (9), 2357-2371. doi: 10.1105/tpc.107.055566

208. Chakraborty, J, Ghosh, P. & Sen S. (2018). Plant Science, 2018, 276, 250-267. doi: 10.1016/j.plantsci.2018.07.014

209. Eulgem, T., Rushton, P.J. & Schmelzer, E. (1999). Early nuclear events in plant defence signalling ：Rapid gene activation by WRKY transcription factors. The EMBO Journal, 18 (17), 4689-4699. doi: 10.1093/emboj/18.17.4689

210.Rushton, P.J., Torres, J.T. & Parniske, M. (1996). Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley Pr1 genes. EMBO 200. Journal, 15 (20), 5690-5700.

211.Turck, F., Zhou, A. & Somssich, I.E.J. (2004). Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to its native promoter and the defense-related gene Pcpr1-1 in parsley. The Plant Cell, 16 (10), 2573-2585. DOI: 10.1105/tpc.104.024810

212. Maleck, K., Levine, A. & Eulgem. T. (2000). The transcriptome of Arabidopsis thaliana during systemic acquired resistance. Nat Genet, 26 (4), 403-410. DOI: 10.1038/82521

213. Mare, C., Mazzucotelli, E. & Crosatti, C. (2004). Hv-WRKY38 ：A new transcription factor involved in cold-and drought-response in barley. Plant Mole Biol, 55 (3), 399-416. DOI: 10.1007/s11103-004-0906-7

214. Cheng, X., Zhao, Y. & Jiang, Q. (2019). Structural basis of dimerization and Dual W-Box DNA recognition by rice WRKY domain. Nucleic Acids Res, 47 (8), 4308 -4318.

215. Colcombet, J. & Hirt, H.J. (2008). Arabidopsis Mapks: A complex signalling

network involved in multiple biological processes. Biochemical Journal, 413 (2), 217-226.

216. Fiil, B.K., Petersen, K. & Petersen, M. (2009). Gene regulation by map kinase cascades. Current Opinion In Plant Biology, 12 (5), 615-621. DOI: 10.1016/j.pbi.2009.07.017

217. Qiu, J.L. & Fiil, B. K. (2008). Arabidopsis map kinase 4 regulates gene expression through transcription factor release in the nucleus. The EMBO Journal, 27 (16), 2214-2221. doi: 10.1038/emboj.2008.147

218. Mao, G., Meng, X. & Liu, Y. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. The Plant Cell, 23 (4), 1639-1653. DOI: 10.1105/tpc.111.084996

219. Guan, Y., Meng, X. & Khanna, R. (2014). Phosphorylation of a WRKY transcription factor by Mapks is required for pollen development and function in Arabidopsis. PLoS Genetics, 10 (5). https://doi.org/10.1371/journal.pgen.1004384

220. Hu, L., Ye, M. & Li, R. (2015). The rice transcription factor WRKY53 suppresses herbivore-induced defenses by acting as a negative feedback modulator of mitogen-activated protein kinase activity. Plant Physiology, 169 (4), 2907-2921. doi: 10.1104/pp.15.01090

221. Wang, Y., Schuck, S. & Wu, J. (2018). A MPK3/6-WRKY33-ALD1 - pipecolic acid regulatory loop contributes to systemic acquired resistance. The Plant Cell, 30 (10), 2480-2494. https://doi.org/10.1105/tpc.18.00547

222. Sarris, P.F., Duxbury, Z. & Huh, S.U. (2015). A plant immune receptor detects pathogen effectors that target WRKY transcription factors. Cell, 161 (5), 1089-1100. DOI: 10.1016/j.cell.2015.04.024

223. Shen, Q.H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., Seki, H., Ulker B., Somssich, I. E. & Paul Schulze-Lefert P. (2007). Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. Science, 315 (5815), 1098-1103. DOI: 10.1126/science.1136372

224. Deslandes, L., Olivier, J. & Theulières, F. (2002). Resistance to Ralstonia solanacearum in Arabidopsis thaliana is conferred by the recessive Rrs1-R gene, a

member of a novel family of resistance genes.Proceedings of the National Academy of Sciences of the USA, 99 (4), 2404-2409. https://doi.org/10.1073/pnas.032485099

225. Deslandes, L., Olivier, J. & Peeters, N. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial Wilt, and Popp2, a type iii effector targeted to the plant nucleus.Proceedings of the National Academy of Sciences of the USA, 100 (13), 8024-8029. https://doi.org/10.1073/pnas.1230660100

226. Vannozzi, A., Wong, D.C.J. & Höll, J. (2018). Combinatorial regulation of stilbene synthase genes by WRKY and Myb transcription factors in grapevine (*Vitis vinifera* L.). Plant Cell Physiology, 59 (5), 1043-1059. DOI: 10.1093/pcp/pcy045

227. Lahiri, A., Venkata, P.S. & Datta, A.J. (2019). Bayesian modeling of plant drought resistance pathway.B MC Plant Biology, 19 (1), 96.

228. Chi, Y., Yang, Y. & Zhou, Y. (2013). Protein-protein interactions in the regulation of WRKY transcription factors. Molecular Plant, 6 (2), 287-300. doi: 10.1093/mp/sst026.

229. Jiao, Z., Sun, J. & Wang, C. (2018). Genome-wide characterization, evolutionary analysis of WRKY genes in cucurbitaceae species and assessment of its roles in resisting to powdery mildew disease. PLoS One, 13 (12).

230. Chen, J., Wang, H. & Li, Y. (2018). Arabidopsis Vq10 interacts with WRKY8 to Modulate basal defense against botrytis cinerea. Journal of Integrative Plant Biology, 60 (10), 956-969. DOI: 10.1111/jipb.12664

231. Jones, H.D., Doherty, A. & Sparks, C.A. (2009). Transient transformation of plants. Methods. Mol. Biol. 513, 131–152. DOI: 10.1007/978-1-59745-427-8_8

232. Andrieu, A., Breitler, J.C., Sire, C., Meynard, D., Gantet, P. & Guiderdoni, E. (2012). An in planta, *Agrobacterium*-mediated transient gene expression method for inducing gene silencing in rice (*Oryza sativa* L.) leaves. Rice 5, 23. DOI: 10.1186/1939-8433-5-23

233. Karlah, N., Robert, H., James, D. & Benjamin, D. (2018). Improving agroinfiltration-based transient gene expression in *Nicotiana benthamiana*. Plant Methods 14, 71.

234. Abel, S. & Theologis, A. (1994). Transient transformation of *Arabidopsis* leaf protoplasts: a versatile experimental system to study gene expression. Plant J. 5, 421–427. DOI: 10.1111/j.1365-313x.1994.00421.x

235. Li, J.F., Park, E., von Arnim, A.G. & Nebenfuhr, A. (2009). The FAST technique: a simplified. Agrobacterium-based transformation method for transient gene expression analysis in seedlings of *Arabidopsis* and other plant species. Plant Methods 5, 6.

236. Krenek, P., Samajova, O., Luptovciak, I., Doskocilova, A., Komis, G. & Samaj, J. (2015). Transient plant transformation mediated by *Agrobacterium tumefaciens*: principles methods and applications. Biotechnol. Adv. 33, 1024–1042. DOI: 10.1016/j.biotechadv.2015.03.012

237. Li, H., Li, K., Guo, Y., Guo, J., Miao, K., Botella, J.R., Song, C.P. & Miao, Y. (2018). A transient transformation system for gene characterization in upland cotton (*Gossypium hirsutum*). Plant Methods, 14, 50.

238. Manavella, P.A. & Chan, R.L. (2009). Transient transformation of sunflower leaf discs via an *Agrobacterium*-mediated method: applications for gene expression and silencing studies. Nat. Protoc. 4, 1699–1707. DOI: 10.1038/nprot.2009.178

239. Mo, R., Huang, Y., Yang, S., Zhang, Q. & Luo, Z. (2015). Development of *Agrobacterium* - mediated transient transformation in persimmon *(Diospyros kaki* Thunb.). Sci. Hortic., 192, 29–37.

240. Francisco, A.R., Beatriz, X., Roberto, T. & Roberto, R. (2015). A simple method for transient transformation of pumpkin (*Cucurbita maxima*) seedlings. Plant Omics, 8, 37–46.

241. Jefferson, R.A. (1987). Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol. Bio. Rep. 5, 387–405.

242. Lee, J. M. (1994). Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. Hort. Science, 29, 235-239. https://doi.org/10.21273/HORTSCI.29.4.235

243. Lee, J. M., Bang H. J. & Ham, H.S (1998). Grafting of vegetables (Grafting and Raising of Seedlings, For Further Development of Horticulture in East Asia). Journal of the Japanese Society for Horticultural Science, 1998, 67, 1098 – 1104. https://doi.org/10.2503/jjshs.67.1098

244. Edelstein, M. (2004). Grafting vegetable - crop plants: pros and cons. Acta Horticulturae, 659, 235 – 238. DOI:10.17660/ActaHortic.2004.659.29

245. Кубрак, С. М. (2021). Щеплення овочевих культур [Електронний ресурс]. – Режим доступу: https://rep.btsau.edu.ua/bitstream/BNAU/6887/1/Shcheplennia_ovochevykh.pdf

246. Yang, P.M., He, S.T., Jiang, L.N., Chen, X.J., Li, Y.F. & Zhou, J.G. (2020). The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose content of different ploidy watermelon (*Citrullus lanatus*). Photosynthetica. 58 (5), 1130-1139. DOI: 10.32615/ps.2020.068

247. Zhou Baoli, Lin Guirong & Li Ningyi (1997). Vegetable grafting cultivation. Beijing: China Agriculture Press, 17-18, 44-45

248. Zheng Qun & Song Weihui (2000). Research progress of vegetable grafting technology at home and abroad (Part 1). Changjiang Vegetables, 8, 1-4

249. Edelstein, M., Tyutyunik, J., Fallik, E., Meir, A. & Tadmor, Y. (2014). Horticultural evaluation of exotic watermelon germplasm as potential rootstocks. Sci Horti, 165, 196-202. doi.org/10.1016/j.scienta.2013.11.010

250. Xu Shengli, Chen Xiaoqing & Chen Qingyun (2004). Physiological characteristics and resistance to fusarium wilt in grafted watermelon plants. Chinese Agricultural Science Bulletin, 20 (2), 149-151

251. Wang Xiqing (2002). Preliminary study on the effect of grafting melon on disease prevention and yield increase. Chinese Watermelon and Melon, 2, 22-23

252. Han Zhiping, Guo Shirong & Zhu Guorong. Effects of rootstock on growth, yield and quality of grafted watermelon. Chinese Vegetables, 2006 (2), 22-26

252. Traka-Mavrona, E., Koutsika-Sotiriou, M. & Pritsa, T. Response of squash

(*Cucurbita* spp.) as rootstock for melon (*Cucumis melo* L.). Scientia Horticulturae, 2000, 83, 353 – 362. DOI:10.1016/S0304-4238(99)00088-6

253. Halit Yetisir & Nebahat Sari (2003). Effect of different rootstock on plant growth, yield and quality of watermelon. Australian Journal of Experimental Agriculture, 43, 1269–1274. DOI:10.1071/EA02095

254. Alan, O., Sen, F. & Duzyaman, E. (2017). The effectiveness of growth cycles on improving fruit quality for grafted watermelon combinations. Food Sci. Technol. 38. Suppl. 1. https://doi.org/10.1590/1678-457x.20817

255. Pina A. & Errea P. (2005). A review of new advances in mechanism of graft compatibility - incompatibility. Scientia Horticulturae, 106, 1 – 11.

256. Rana Shahzad Noor, Zhi Wang, Muhammad Umair, Muhammad Yaseen, Muhammad Ameen, Shoaib-Ur Rehman, Muzammil Usman Khan, Muhammad Imran, Waqar Ahmed & Yong Sun (2019). Interactive Effects of Grafting Techniques and Scion-Rootstocks Combinations on Vegetative Growth, Yield and Quality of Cucumber (*Cucumis sativus* L.). Agronomy, 9 (6), 288. https://doi.org/10.3390/agronomy9060288

257. Chen Liping, Song Zengjun & Ma Xingzhuang (2004). Effect of grafting on quality of cucumber in solar greenhouse. Journal of Northwest Agricultural Sciences, 13 (2), 170-171. DOI: 10.17660/ActaHortic.2007.761.47

258. Li Hongli, Yu Xianchang & Wang Huasen (2005). Effects of grafting and grafting rootstock on fruit quality of cucumber. Journal of Northwest Agricultural Sciences, 14 (1), 129-132.

259. Zhong, Y. Q. & Bie, Z. L. (2007). Effects of grafting on the growth and quality of cucumber fruits. Acta Hortic., 761, 341-347. DOI: 10.17660 /ActaHortic.2007.761.47

260. Yetisir, H. & Sari, N. (2004). Effect of hypocotyl morphology on survival rate and growth of watermelon seedlings grafted on rootstocks with different emergence performance at various temperatures, Turkish Journal of Agriculture and Forestry, 28, 231 -237.

261. Cao Jianhua, Lin Weifu & Chen Junming (2005). A review of the affinity between rootstock and scion grafting. Journal of Tropical Agriculture, 25 (4), 64-69.

262. Ruiz, J. M., Belakbir, A., Lopez-Cantarero, I. & Romero, L. (1997). Leaf - macronutrient content and yield in grafted melon plants. A model to evaluate the influence of rootstock genotype. Scientia Horticulturae, 71, 227-234. https://doi.org/10.1016/S0304-4238(97)00106-4

263. Liu, H. Y., Zhu, Z. I., Qian, Q. Q. & Ge, Z. P. (2004). The effects of different rootstocks on the sugar metabolism and related enzyme activities in small and early-maturing watermelon during fruit development. *Acta Horticulturae*, (31), 47-52.

264. Zhang Yanpeng, Yu Xianchang & Zhang Zhenxian (2004). Photosynthetic characteristics and protective enzyme activities of grafted cucumber in solar greenhouse. Chinese Journal of Horticulture, 31 (1), 94-96.

265. Zhang Hongmei, Huang Danfeng & Ding Minget (2005). Changes of three enzyme activities during the healing process of watermelon grafts with different seedling age scions. Biological Physiology Communication, 41 (3), 302- 304.

266. Zeng Yi'an, Zhu Yuelin & Huang Huixin (2005). Studies on photosynthetic characteristics, hormone content and soluble protein in grafted cucumber leaves. Journal of Nanjing Agricultural University, 28 (1), 16-19.

267. Ruiz, J. M. & Romero, L. (1999). Nitrogen efficiency and metabolism in grafted melon plants. Scientia Horticulturae, 81, 113-123. https://doi.org/10.1016/S0304-4238(98)00200-3

268. Pulgar, G., Vilora, G., Moreno, D. A. & Romero, L. (2000). Improving the mineral nutrition in grafted watermelon plants: nitrogen metabolism. Biologia Plantarum, 43, 607-609. DOI: 10.1023/A:1002856117053

269. Liu Huiying, Zhu Zhujun, Qian Qiongqiuet (2004). Effects of rootstock on sugar metabolism and related enzyme activities in small early maturing watermelon fruits. Acta Horticulturae Sinica, 31 (1), 47-52.

270. Yang Lifei, Zhu Yuelin & Hu Chunmeiet (2005). Study on growth dynamics and leaf physiological and biochemical characteristics of grafted watermelon under

NaCl stress. Southwest China Journal of Agricultural Sciences, 18 (4), 439-443.

271. Park, C. Y., Lee J. H. & Yoo, J. H. (2005). WRKY group IId transcription factors interact with calmodulin. FEBS Letters, 579 (6), 1545-1550. doi: 10.1016/j.febslet.2005.01.057.

272. Cohen, R., Burger, Y., Horev, C., Porat, A. & Edelstein, M. (2005). Performance of Galia-type melons grafted on to Cucurbita rootstock in Monosporascus cannonballus-infested and non-infested soils. Annals of Applied Biology, 146, 381-387. https://doi.org/10.1111/j.1744-7348.2005.040010.x

273. Xu Shengli, Chen Xiaoqing & Chen Qingyun (2004). Physiological characteristics and resistance to fusarium wilt in grafted watermelon plants. Chinese Agricultural Science Bulletin, 20 (2), 149-151.

274. Masuda, M., Nakamura, T. & Gomi, K. (1981). Studies on the characteristics of nutrient absorption of rootstocks in grafting fruit vegetable. Bulletin Faculty of Agriculture, 27, 179-186.

275.Wang Yuyan, Jia Weiguo & Shen Sile (1995). Study on physiological effects of different rootstocks on grafted cucumber. Chinese Vegetables, 2, 31-34.

276. Colla, G., Suãrez, C.M.C., Cardarelli, M. & Rouphael, Y (2010). Improving nitrogen use efficiency in melon by grafting. HortScience, 45, 559-565.

277. Yu Xianchang (1997). Cold resistance of grafted cucumber seedlings. Doctoral Dissertation of Nanjing Agricultural University, 13-24.

278. Yu Xianchang & Wang Lijiang (1998). Research and application of vegetable grafting. Journal of Shandong Agricultural University, 2, 249-256.

279. Chen Guilin, Ie Lanchun & Li Jianwenet (2000). Effects of low temperature stress on photosynthetic characteristics of grafted zucchini seedlings. Journal of Shanghai Agricultural Sciences, 1, 42 – 45.

280. Wang Yuyan, Jia Weiguo & Shen Sileet (1995). Study on physiological effects of different rootstocks on grafted cucumber. Chinese Vegetables, 2, 31-34.

281.Yang Shijie & Lu Shanfa (1995). Study on the basic theory of plant grafting, Biology Bulletin, 30 (9), 10-12.

282. Colla, G., Rouphael, Y., Cardarelli, M., Salerno, A. & Rea, E. (2010). The effectiveness of grafting to improve alkalinity tolerance in watermelon. Environ.

Exp. Bot, 68, 283-291. https://doi.org/10.1016/j.envexpbot.2009.12.005

283. Shi Yuelin, Liu Peiying, Luo Qingxiet (1995). Effect of pumpkin anvil with blac k seed on salt resistance of cucumber. Journal of Southwest Agricultural University, 17 (3), 232-236.

284. Zhang Yunqi, Liu Shiqi & Yang Fengjuan (2003). Study on screening of salt-tolerant watermelon rootstock and its salt-tolerant mechanism. Journal of Northwest Agricultural Sciences, 12 (4), 105-108.

285. Yunqi Zhang, Shiqi Liu & Haibo Wang (2004). Effect of salt-tolerant rootstock grafting on salt-resistant characteristics of watermelon seedlings. Journal of Shanghai Agricultural Sciences, 20 (3), 62 – 64.

286. Colla, G., Rouphael, Y., Leopardi, C. & Bie, Z. (2010). Role of grafting invegetable crops grown under saline conditions. Sci. Hort. 127, 147-155.

287. Yue Qing, Miao Yi & Fan Sanjiang (1999). Effect of different rootstocks on grafting effect of watermelon. Journal of Shanxi Normal University (Natural Science Edition), 1, 53-55.

288. Lu Wenjing. Study on technology of grafting disease resistance and increasing yield of Muskmelon with thin skin (2002). Liaoning Agricultural Sciences, 1, 16-20.

289. Halit Yetışır, Nebahat Sari & Seral Yücel **(**2003). Rootstock resistance to Fusarium wilt and effect on watermelon fruit yield and quality. Phytoparasitica 31(2), 163-169. DOI:10.1007/BF02980786

290. Zeng Yi'an, Zhu Yuelin & Huang Huixin (2004). Effects of pumpkin rootstock with black seed on fruit bearing, disease resistance and nutrient content of cucumber. Journal of Plant Resources and Environment, 13 (4), 15-19.

291. Miguel, A., Maroto, J.V. & Bautista, A. S. (2004). The grafting of triploid watermelon is an advantageous alternative to soil fumigation by methy bromide for control of Fusarium wit. Scientia Horticulturae, 103, 9-17. DOI:10.1016/j.scienta.2004.04.007

292. Crinò, P., Lo Bianco, C., Rouphael, Y., Colla, G., Saccardo, F. & Paratore, A. (2007). Evaluation of rootstock resistance to fusarium wilt and gummy stem blight

and effect on yield and quality of a grafted ‘Inodorus’ melon. Hort Sci., 42, 521-525. https://doi.org/10.21273/HORTSCI.42.3.521

293. Шерстюк, М.Ю., Скляр, В.Г., Скляр, Ю.Л. & Хе Сунтао (2019). Комплексний популяційний аналіз як напрямок сучасних біолого-екологічних досліджень. Вісник Сумського національного аграрного університету. Серія «Агрономія і біологія», 3 (37), 61-67.

294. Злобін, Ю.А., Скляр, В.Г. & Клименко, Г.О. (2022). Біологія та екологія фітопопуляцій. Суми: Унів. книга, 512.

295. Bondarieva L.M., Kyrylchuk K.S., Skliar V.H., Tikhonova O.M., Zhatova H.O. & Bashtovyi M.G. (2019). Population dynamics of the typical meadow species in the conditions of pasture digression in flooded meadows. Ukrainian Journal of Ecology. 9 (1), 204–211.

296. Скляр В.Г. (2013). Динаміка віталітетних параметрів лісоутворювальних видів Новгород-Сіверського Полісся: теоретичні засади та способи оцінки. Укр. ботан. журн., 70, 5, 624-630.

297. Zhong, Y., Qi, X., Chen, J., Li, Z., Bai, D., Wei, C. & Fang, J. (2019). Growth and physiological responses of four kiwifruit genotypes to salt stress and resistance evaluation Journal of Integrative Agriculture, 18(1), 83–95.

298. Yaming Fang, Gang Lu & Libei Xiong (2007). Determination of Lead, cadmium, arsenic and mercury in food by microwave digestion. Environmental and Occupational Medicine, 24 (2), 212-114.

299. Liu Hui, Qian Qiang & Jin Wei (2016). Determination of 13 Metallic Elements in Fruits and Vegetables by inductively coupled Plasma Mass Spectrometry. Journal of Food Safety and Quality Inspection, 7 (11), 4672-4676.

300. Li Wenzhi, Wang Chunlan & Lin Pei (2013). Determination and analysis of 6 Heavy Metal Elements in vegetables and Fruits in Fuzhou. Chinese Journal of Health Laboratory, 23 (2), 463-465. https://doi.org/10.3390/horticulturae8111034

301. Wei Rufeng & Deng Yiping (2018) Application of Microwave digestion technology in food analysis. Popular Science and Technology, 20 (3), 23-25.

302.Yan Lamei, Zhao Jing & Wang Diqiang (2009). Determination of total arsenic in liquor by microwave digestion-atomic fluorescence spectrometry. Wine Science

and Technology, 5, 107-109.

303. Tang Lianxian, Wu Xiao & Zheng Shaocheng (2008). Hydride generation-atomic fluorescence spectrometry. Chemical Analysis and Metrology, 17 (2), 34-36. https://doi.org/10.1021/ac020695h

304. Calin, C, Scaeteanu, G. & Pele, M. (2012). Assessment of copper content in wines from tohani-dealu mare by flame atomic absorption spectrometry. Revistade Chimie, (63), 1062-1064.

305. Rodrguez-Solana, R., Salgado, J.M. & Domnguez, J.M. (2014). Assessment of minerals in aged grape marc distillates by FAAS/FAES and ICP-MS. Characterization and safetye valuation. Food Control, 35 (1), 49-55. DOI:10.1016/J.FOODCONT.2013.06.031

306.Tariba, B., Pizent, A. & Kljakovia-Gaspic, Z. (2011). Determination of lead in croatian wines by graphite furnace atomic absorption spectrometry, Arhiv za Higijenu Rada I Toksikologiju-archives of Industrial Hygiene and Toxicology, 62, 25-31. doi: 10.2478/10004-1254-62-2011-2073

307. Xiao-Dong Pan, Jun Tang, Qing Chen, Ping-Gu Wu & Jian-Long Han (2013). Evaluation of direct sampling method for trace elements analysis in Chinese rice wine by ICP-OES. Eur Food Technol, 23, 531-535. Doi: 10.1007/s00217-012-1888-3

308. Catarino, S., Curvelo-Garcia, A. S. & Desousa, R. B. (2006). Measurements of contaminant elements of wines by inductively coupled plasma-mass spectrometry: A comparison of two calibration approaches. Talanta, 70, 1073-1080. DOI:10.1016/j.talanta.2006.02.022

309. Castineira, M., Brandt, R. & Vonbohlen, A. (2001). Development of a procedure for the multi-element determination of trace elements in wine by ICP-MS. Fresenius J Anal Chem, 370, 553-558. DOI: 10.1007/s002160100862

310. Selih, V.S., Sala, M. & Drgan, V. (2014). Multi-element analysis of wines by ICP-MS and ICP-OES and their classification according to geographical origin in Slovenia. Food Chem, 153 (15), 414-423. doi: 10.1016/j.foodchem.2013.12.081

311. Yang, P.M., He, S.T., Jiang, L.N., Chen, X.J., Li, Y.F., & Zhou, J.G. (2020). The effects of pumpkin rootstock on photosynthesis, fruit mass, and sucrose

content of different ploidy watermelon (Citrullus lanatus). Photosynthetica. 58 (5), 1130-1139. DOI: 10.32615/ps.2020.068

312.Hassell, R.L., Memmott, F. & Liere, D.G. (2008). Grafting methods for watermelon production. Hortscience 43, 1677-1679.

313. Peng-Ming Yang & Song-Tao He (2022). The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*). Horticultural Science (Prague), 1-9. https://doi.org/10.17221/108/2021-HORTSCI

314. Baker, N.R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. – Annu. Rev. Plant Biol. 59, 89-113. doi: 10.1146/annurev.arplant.59.032607.092759

315. Liu, J., Guo, S. & He, H. (2013). Dynamic characteristics of sugar accumulation and related enzyme activities in sweet and non-sweet watermelon fruits. Acta Physiol. Plant. 35, 3213-3222. DOI:10.1007/s11738-013-1356-0

316. Gao, Z. & Schaffer, A.A. (1999). A novel alkaline alpha-galactosidase from melon fruit with a substrate preference for raffinose. – Plant Physiol., 119, 979-988. DOI: 10.1104/pp.119.3.979

317. Miron, D. & Schaffer, A.A. (1991). Sucrose phosphate synthase, sucrose synthase, and invertase activities in developing fruit of Lycopersicon esculentum Mill. and the sucrose accumulating Lycopersicon hirsutum Humb. and Bonpl. – Plant Physiol., 95, 623-627. doi: 10.1104/pp.95.2.623

318. Hubbard, N.L., Huber, S.C. & Pharr, D.M. (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol., 91, 1527-1534. DOI: 10.1104/pp.91.4.1527

319. Lowell, C.A., Tomlinson, P.T. & Koch, K.E. (1989). Sucrose-metabolizing enzymes in transport tissues and adjacent sink structures in developing citrus fruit. Plant Physiol., 90, 1394-1402. DOI: 10.1104/pp.90.4.1394

320. Ulker, B. & Somssich, I.E. (2004). WRKY transcription factors: from DNA bioding towards biological towards biological function. Current Opinion in Plant Biology, 7 (5), 491-498. DOI: 10.1016/j.pbi.2004.07.012

321. El-Gebali, S., Mistry, J. & Bateman, A. (2019). The Pfam protein families database in 2019. Nucleic Acids Research, 47, 427-432. DOI: 10.1093/nar/gky995

322. Finn, R. D., Clements, J. & Eddy, S. R. (2011). HMMER web server: Interactive sequence similarity searching. Nucleic Acids Research, 39 (Web Server issue), 29-37. doi: 10.1093/nar/gkr367

323. Chou, K.C. & Shen, H. B. (2010). A new method for predicting the subcellular localization of eukaryotic proteins with both single and multiple sites: Euk-mPLoc 2.0. PLOS ONE, 5 (4).

324. Eulgem, T, Rushton, P.J. & Robatzek, S. (2000). The WRKY superfamily of plant transcription factors. Trends in Plant Science, 5 (5), 199-206. doi: 10.1016/s1360-1385(00)01600-9.

325. Kumar, S., Stecher, G. & Li, M. (2018). MEGA X: Molecular evolutionary genetics analysis across computing plat- forms. Molecular Biology and Evolution, 35 (6), 1547- 1549. DOI: 10.1093/molbev/msy096

326. Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39 (4), 783-791. https://doi.org/10.2307/2408678

327. Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4 (4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

328. Nei, M. & Kumar, S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York, 235-296.

329. He, Z., Zhang, H. & Gao, S. (2016). Evolview v2: An online visualization and management tool for customized and annotated phylogenetic trees. Nucleic Acids Research, 44 (W1), 236-241. doi: 10.1093/nar/gkw370

330. Bailey, T. L., Williams, N. & Misleh, C. (2006). MEME: Discovering and analyzing DNA and protein sequence motifs. Nucleic Acids Research, 34 (Web Server issue), 369-373. doi: 10.1093/nar/gkl198

331. Xuejin Chena, Songtao He, Lina Jiang, Xinzheng Li, Weili Guo, Bihua Chena, Junguo Zhoua & Viktoriia Skliar (2021). An efficient transient transformation system for gene function studies in pumpkin (*Cucurbita moschata* D.). Scientia Horticulturae, 1, 1-12. DOI:10.1016/j.scienta.2021.110028

332. Царенко, О.М., Злобін, Ю.А., Скляр, В.Г. & Панченко, С.М. (2000). Комп'ютерні методи в сільському господарстві та біології. Суми: Університетська книга, 203.

333. Ali, M.A, Azeem, F. & Nawaz, M.A. (2018). Transcription factors WRKY1l and WRKY17 are involved in abiotic stress responses in *Arabidopsis*. J Plant Physiol., 226, 12-21.

334. Liu, X., Song, Y. & Xing F. (2016). *GhWRKY25*, a group I*WRKY* gene from cotton, confers differential tolerance to abiotic and biotic stresses in transgenic *Nicotiana benthamiana*. Protoplasma, 253, 1265-1281.

335. Jiang, Y., Qiu, Y. & Hu, Y. (2016). Heterologous expression of AtWRKY57 confers drought tolerance in *Oryza sativa*. Front Plant Sci, 7, 145.

336. Zhou, Q.Y., Tian, A.G. & Zou, H.F. (2008). Soybean WRKY type transcription factor genes, *GmWRKY13*, *GmWRKY21*, and *GmWRKY54*. confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. Plant Biotechnol J, 6, 486-503.

337. Lai, Z., Vinod, K. & Zheng, Z. (2008). Roles of *Arabidopsis* WRKY3 and WRKY4 transcription factors in plant responses to pathogens. BMC Plant Biol, 8, 68.

338. Schluttenhofer, C., Pattanaik, S. & Patra, B. (2014). Analyses of *Catharanthus roseus* and *Arabidopsis thaliana* WRKY transcription factors reveal involvement in jasmonate signaling. BMC Genomics, 15, 502.

339. Fu, Q.T. & Yu, D.Q. (2010). Expression profiles of *AtWRKY25*, *AtWRKY26* and *AtWRKY33* under abiotic stresses. Hereditas, 32, 848-856.

340. Robatzek, S. & Somssich, I.E. (2001). A new member of the *Arabidopsis* WRKY transcription factor family, *AtWRKY6* is associated with both senescence- and defence-related processes. Plant J, 28, 123-133.

341. Jiang, Y., Liang, G. & Yu, D. (2012). Activated expression of WRKY57 confers drought tolerance in *Arabidopsis*. Mol Plant, 5, 1375-1388.

342. Van Aken, O., Zhang, B. & Law, S. (2013). AtWRKY40 and AtWRKY63 modulate the expression of stress-responsive nuclear genes encoding mitochondrial and chloroplast proteins. Plant Physiol, 162, 254-271.

343. Chen, C. & Chen, Z. (2002). Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced *Arabidopsis* transcription factor. Plant Physiol, 129, 706-716.

344. Xie, Z.W., Wang, L.J. & Chen, J.Y. (2016). Studies on WRKY transcription factors and their biological functions in plants. J Agric Sci Tech, 18, 46-54.

345. Goddemeier, M.L., Wulff, D. & Feix, G. (1998). Root-specific expression of a *Zea mays* gene encoding a novel glycine-rich protein, zmGRP3. Plant Mol Biol, 36, 799-802.

346. Nolan, K.E., Irwanto, R.R. & Rose, R.J. (2003). Auxin up-regulates *MtSERK1* expression in both *Medicago truncatula* root forming and embryogenic cultures. Plant Physiol., 133, 218-230.

347. He Songtao, Zhou Junguo & Skliar V.G. (2019). The problem of soil salinization and the role of genetic engineering in increasing the salt tolerance of plants. Матеріали Міжнародної науково-практичної конференції, присвяченої 90-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (24- 25 травня 2019 р.), 13.

348. Chen, G.H., Wen, Y., Yang, L.F., Cai, J.Y. & Zhu, Y.L. (2014). Overexpression of StNHX1, a novel vacuolar Na^+/H^+ antiporter gene from Solanum torvum, enhances salt tolerance in transgenic vegetable soybean. Hortic. Environ. Biotechnol., 55, 213–221.

349. Chou, K.C. & Shen, H.B. (2007). Cell-ploc: a package of web servers for predicting subcellular localization of proteins in various organisms. Nat. Protoc. 3, 153–162.

350. Cartmilla, D.L., Alarcón, A., Volderc, A., Valdez-Aguilard, L.A., Arnoldc, M.A. & Cartmille, A.D. (2012): Arbuscular mycorrhizal fungi alleviate growth of *Ulmus parvifolia* Jacq. at suboptimal planting depths. Scientia Horticulturae, 144, 74–80.

351. Mathur, S., Tomar, R.S. & Jajo, A. (2019). Arbuscular mycor- rhizal fungi (AMF) protects photosynthetic apparatus of wheat under drought stress. Photosynthesis Research, 139, 227–238.

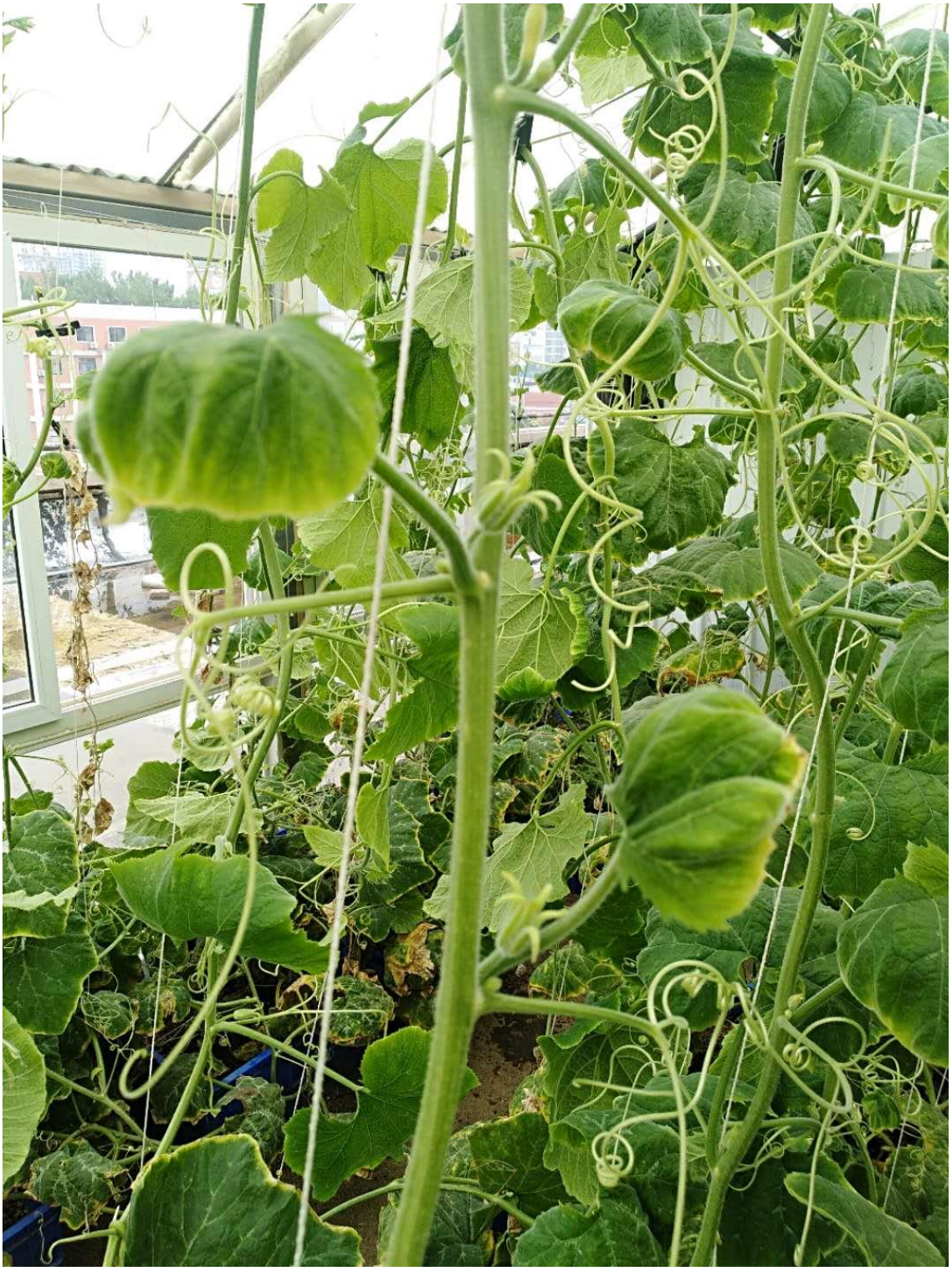
352. Kazadi, A.T., Lwalaba, J.L.W., Ansey, B.K., Muzulukwau, J.M., Katabe, G.M., Karul, M.I., Baert, G., Haesaert, G. & Mundende, R.P.M. (2022): Effect of

phosphorus and arbuscular mycorrhizal fungi (AMF) inoculation on growth and productivity of maize (*Zea mays* L.) in a tropical ferralsol. Gesunde Pflanzen, 74, 159–165.

APPENDICES

APPENDIX A

PLANTS THAT HAVE UNDERGONE SALT STRESS



APPENDIX B

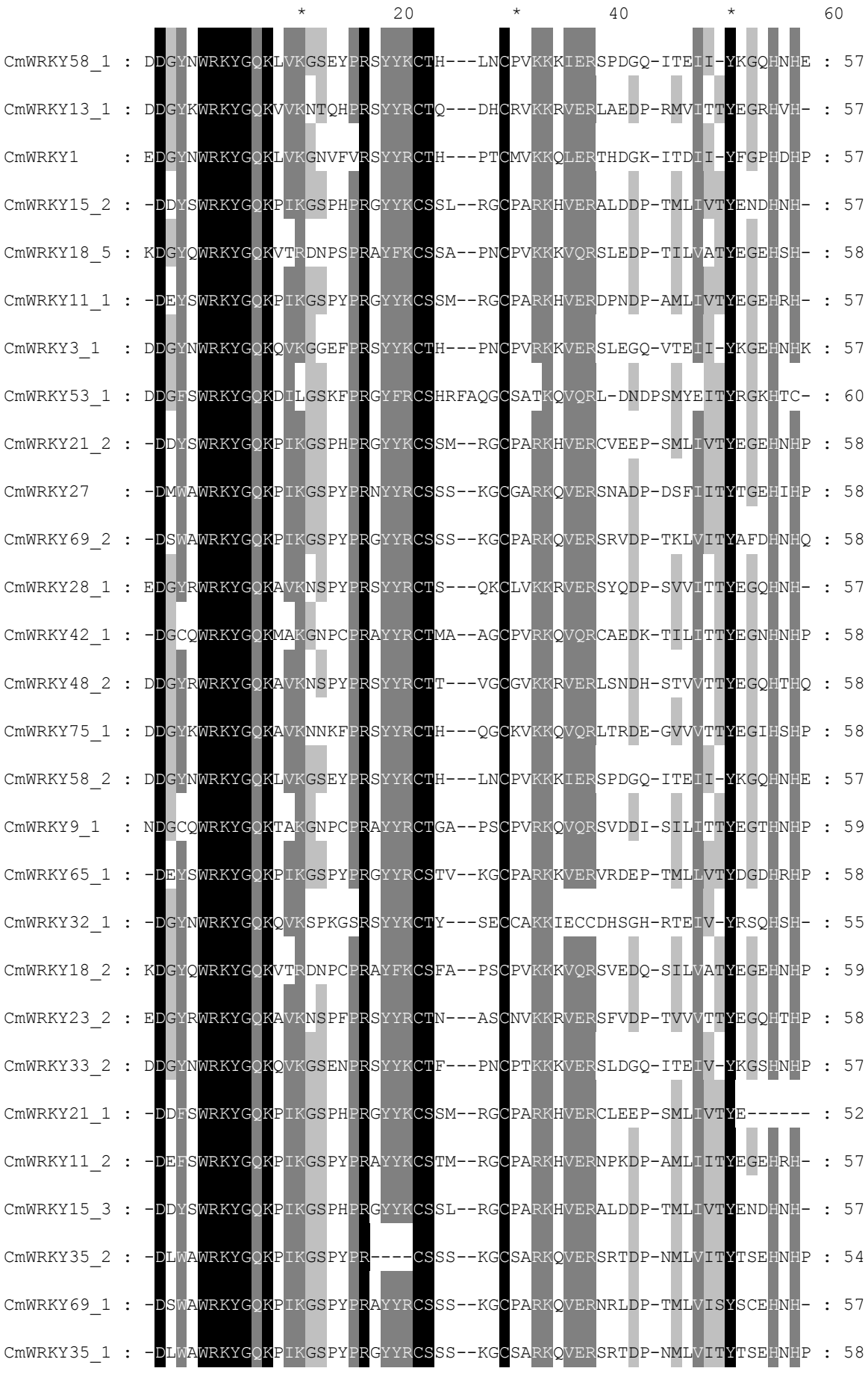
Appendix B.1. An example of the results of morphometric analysis (indicators of stem diameter (unit:mm))

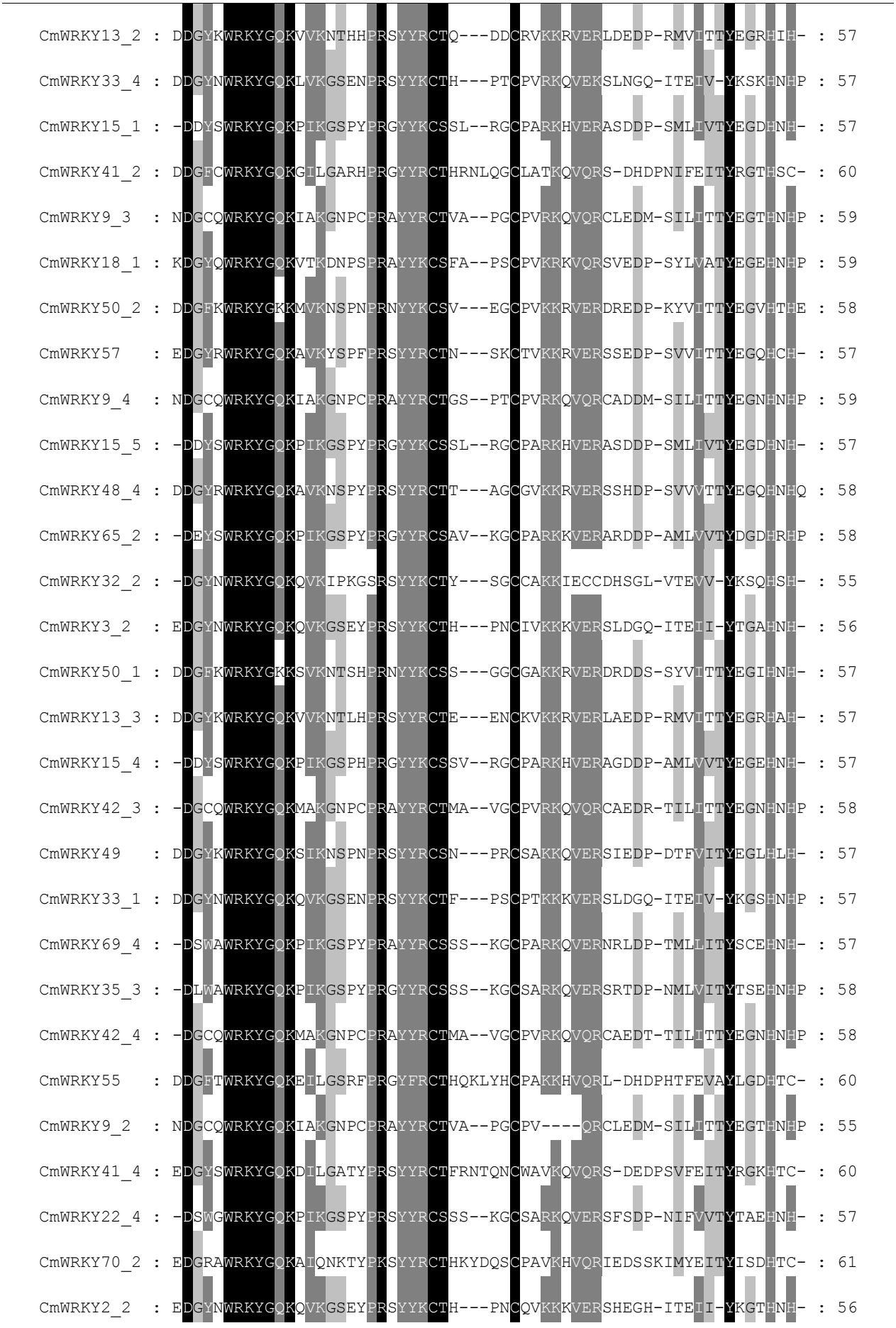
CkMiben12	CkMiben11	CkMiben10	CkMiben9	CkMiben8	CkMiben7	CkMiben6	CkMiben5	CkMiben4	CkMiben3	CkMiben2	CkMiben1
9.93	8.21	9.64	9.04	9.34	8.30	8.70	8.30	9.17	8.88	8.47	9.20
CkYanzhen12	CkYanzhen11	CkYanzhen10	CkYanzhen9	CkYanzhen8	CkYanzhen7	CkYanzhen6	CkYanzhen5	CkYanzhen4	CkYanzhen3	CkYanzhen2	CkYanzhen1
8.27	8.73	8.26	9.03	7.81	8.03	7.67	7.98	7.43	7.81	8.79	8.60
60Miben12	60Miben11	60Miben10	60Miben9	60Miben8	60Miben7	60Miben6	60Miben5	60Miben4	60Miben3	60Miben2	60Miben1
7.68	8.42	7.82	9.20	8.15	8.75	8.43	8.28	7.78	8.28	9.27	9.04
60Yanzhen12	60Yanzhen11	60Yanzhen10	60Yanzhen9	60Yanzhen8	60Yanzhen7	60Yanzhen6	60Yanzhen5	60Yanzhen4	60Yanzhen3	60Yanzhen2	60Yanzhen1
6.78	8.47	7.90	8.23	8.53	7.80	7.68	7.51	7.00	7.84	7.02	7.58
120Miben12	120Miben11	120Miben10	120Miben9	120Miben8	120Miben7	120Miben6	120Miben5	120Miben4	120Miben3	120Miben2	120Miben1
6.95	8.33	7.75	7.9	10.5	8.06	8.02	7.30	8.37	8.47	7.90	8.36
120Yanzhen12	120Yanzhen11	120Yanzhen10	120Yanzhen9	120Yanzhen8	120Yanzhen7	120Yanzhen6	120Yanzhen5	120Yanzhen4	120Yanzhen3	120Yanzhen2	120Yanzhen1
7.81	7.21	7.21	6.21	7.62	7.62	9.85	8.23	7.24	7.86	7.77	6.97

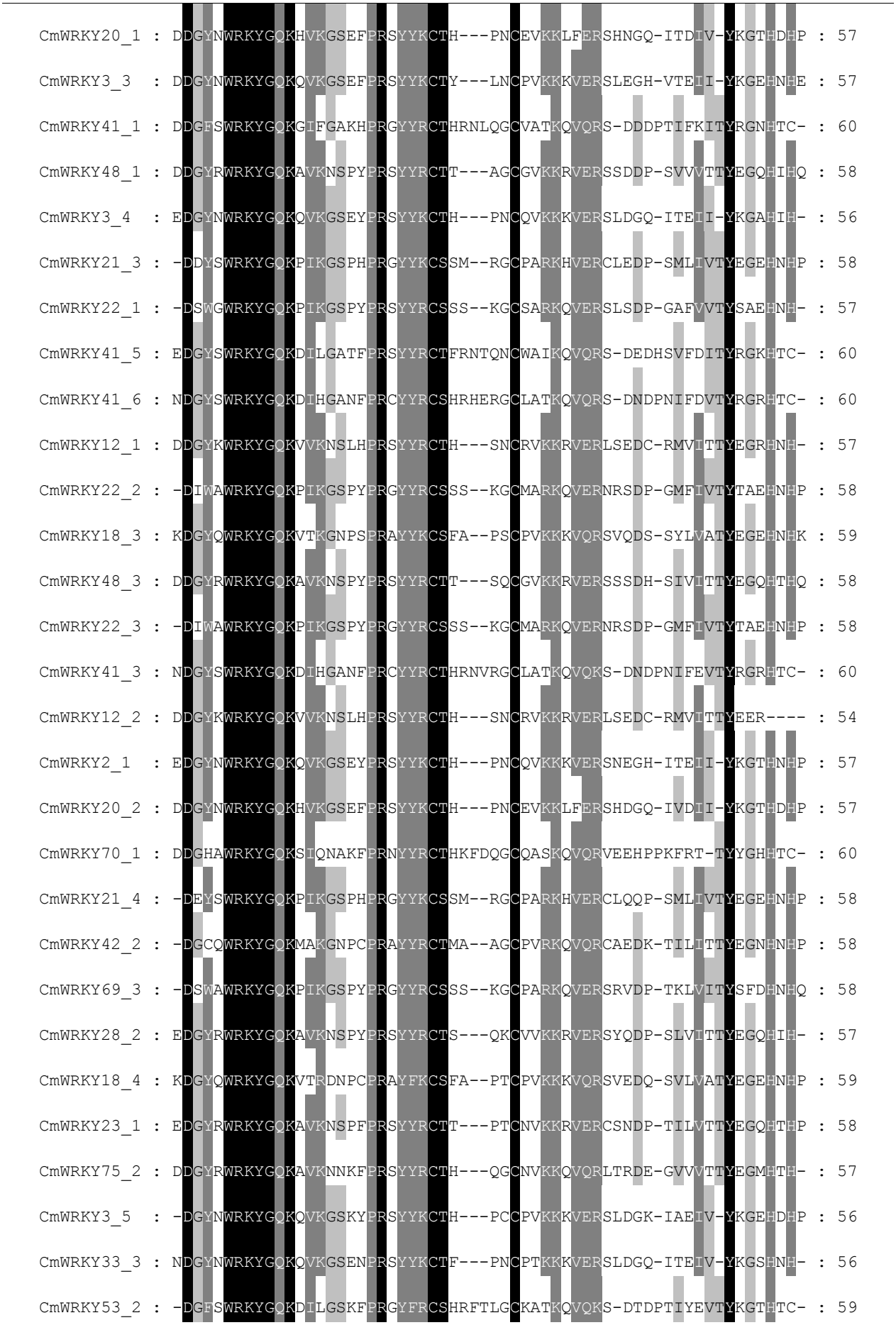
Appendix B.2. An example of the results of morphometric analysis (indicators stem long (unit:cm))

CkMiben12	CkMiben11	CkMiben10	CkMiben9	CkMiben8	CkMiben7	CkMiben6	CkMiben5	CkMiben4	CkMiben3	CkMiben2	CkMiben1
13	11	10	12	13	10.5	11.5	11	9	11.5	12	11
CkYanzhen12	CkYanzhen11	CkYanzhen10	CkYanzhen9	CkYanzhen8	CkYanzhen7	CkYanzhen6	CkYanzhen5	CkYanzhen4	CkYanzhen3	CkYanzhen2	CkYanzhen1
14	14	14.5	16	13	14	14.5	15.5	12	13.5	12.5	13.5
60Miben12	60Miben11	60Miben10	60Miben9	60Miben8	60Miben7	60Miben6	60Miben5	60Miben4	60Miben3	60Miben2	60Miben1
9	12	9.5	10	13	11	11	13	11	12.5	9	10
60Yanzhen12	60Yanzhen11	60Yanzhen10	60Yanzhen9	60Yanzhen8	60Yanzhen7	60Yanzhen6	60Yanzhen5	60Yanzhen4	60Yanzhen3	60Yanzhen2	60Yanzhen1
10.5	14	14	15	13	13	12.5	7.5	12.5	12.5	10.5	10
120Miben12	120Miben11	120Miben10	120Miben9	120Miben8	120Miben7	120Miben6	120Miben5	120Miben4	120Miben3	120Miben2	120Miben1
8.5	11	14	9	10.5	8	12	13	9.5	8.5	9.5	9.5
120Yanzhen12	120Yanzhen11	120Yanzhen10	120Yanzhen9	120Yanzhen8	120Yanzhen7	120Yanzhen6	120Yanzhen5	120Yanzhen4	120Yanzhen3	120Yanzhen2	120Yanzhen1
10	9.5	11.5	9	10	10	11	9	10.5	11	10.5	6

APPENDIX C
ANALYSIS OF CONSERV ATIVE SEQUENCE OF WRKY TRANSCRIPTION FACTORS IN PUMPKIN




CmWRKY13_2 : DDGYKWRKYGQKVVKNTHHPRSYYRCTQ---DDCRVKKRVERLDEDP-RMVITTYEGRHIH- : 57
CmWRKY33_4 : DDGYNWRKYGQKLVKGSENPRSYYKCTH---PTCPVRKQVEKSLNGQ-ITEIV-YKSKHNHP : 57
CmWRKY15_1 : -DDYSWRKYGQKPIKGSPYPRGYYKCSSL--RGCPARKHVERASDDP-SMLIVTYEGDHNH- : 57
CmWRKY41_2 : DDGFCWRKYGQKGILGARHPRGYYRCTHRNLQGCLATKQVQRS-DHDPNIFEITYRGTHSC- : 60
CmWRKY9_3 : NDGCQWRKYGQKIAKGNPCPRAYYRCTVA--PGCPVRKQVQRCLEDM-SILITTYEGTHNHP : 59
CmWRKY18_1 : KDGYQWRKYGQKVTKDNPSPRAYYKCSFA--PSCPVKRKVQRSVEDP-SYLVATYEGEHNHP : 59
CmWRKY50_2 : DDGFKWRKYGKKMVKNSPNPRNYYKCSV---EGCPVKKRVERDREDP-KYVITTYEGVHTHE : 58
CmWRKY57 : EDGYRWRKYGQKAVKYSPFPRSYYRCTN---SKCTVKKRVERSSEDP-SVVITTYEGQHCH- : 57
CmWRKY9_4 : NDGCQWRKYGQKIAKGNPCPRAYYRCTGS--PTCPVRKQVQRCADDM-SILITTYEGNHNHP : 59
CmWRKY15_5 : -DDYSWRKYGQKPIKGSPYPRGYYKCSSL--RGCPARKHVERASDDP-SMLIVTYEGDHNH- : 57
CmWRKY48_4 : DDGYRWRKYGQKAVKNSPYPRSYYRCTT---AGCGVKKRVERSSHDP-SVVVTTYEGQHNHQ : 58
CmWRKY65_2 : -DEYSWRKYGQKPIKGSPYPRGYYRCSAV--KGCPARKKVERARDDP-AMLVVTYDGDHRHP : 58
CmWRKY32_2 : -DGYNWRKYGQKQVKIPKGSRSYYKCTY---SGCCAKKIECCDHSGL-VTEVV-YKSQHSH- : 55
CmWRKY3_2 : EDGYNWRKYGQKQVKGSEYPRSYYKCTH---PNCIVKKKVERSLDGQ-ITEII-YTGAHNH- : 56
CmWRKY50_1 : DDGFKWRKYGKKSVKNTSHPRNYYKCSS---GGCGAKKRVERDRDDS-SYVITTYEGIHNH- : 57
CmWRKY13_3 : DDGYKWRKYGQKVVKNTLHPRSYYRCTE---ENCKVKKRVERLAEDP-RMVITTYEGRHAH- : 57
CmWRKY15_4 : -DDYSWRKYGQKPIKGSPHPRGYYKCSSV--RGCPARKHVERAGDDP-AMLVVTYEGEHNH- : 57
CmWRKY42_3 : -DGCQWRKYGQKMAKGNPCPRAYYRCTMA--VGCPVRKQVQRCAEDR-TILITTYEGNHNHP : 58
CmWRKY49 : DDGYKWRKYGQKSIKNSPNPRSYYRCSN---PRCSAKKQVERSIEDP-DTFVITYEGLHLH- : 57
CmWRKY33_1 : DDGYNWRKYGQKQVKGSENPRSYYKCTF---PSCPTKKKVERSLDGQ-ITEIV-YKGSHNHP : 57
CmWRKY69_4 : -DSWAWRKYGQKPIKGSPYPRAYYRCSSS--KGCPARKQVERNRLDP-TMLLITYSCEHNH- : 57
CmWRKY35_3 : -DLWAWRKYGQKPIKGSPYPRGYYRCSSS--KGCSARKQVERSRTDP-NMLVITYTSEHNHP : 58
CmWRKY42_4 : -DGCQWRKYGQKMAKGNPCPRAYYRCTMA--VGCPVRKQVQRCAEDT-TILITTYEGNHNHP : 58
CmWRKY55 : DDGFTWRKYGQKEILGSRFPRGYFRCTHQKLYHCPAKKHVQRL-DHDPHTFEVAYLGDHTC- : 60
CmWRKY9_2 : NDGCQWRKYGQKIAKGNPCPRAYYRCTVA--PGCPV----QRCLEDM-SILITTYEGTHNHP : 55
CmWRKY41_4 : EDGYSWRKYGQKDILGATYPRSYYRCTFRNTQNCWAVKQVQRS-DEDPSVFEITYRGKHTC- : 60
CmWRKY22_4 : -DSWGWRKYGQKPIKGSPYPRSYYRCSSS--KGCSARKQVERSFSDP-NIFVVTYTAEHNH- : 57
CmWRKY70_2 : EDGRAWRKYGQKAIQNKTYPKSYYRCTHKYDQSCPAVKHVQRIEDSSKIMYEITYISDHTC- : 61
CmWRKY2_2 : EDGYNWRKYGQKQVKGSEYPRSYYKCTH---PNCQVKKKVERSHEGH-ITEII-YKGTHNH- : 56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CmWRKY3_3 : DDGYNWRKYGQKQVKGSEFPRSYYKCTY---LNCPVKKKVERSLEGH-VTEII-YKGEHNHE : 57
CmWRKY41_1 : DDGFSWRKYGQKGIFGAKHPRGYYRCTHRNLQGCVATKQVQRS-DDDPTIFKITYRGNHTC- : 60
CmWRKY48_1 : DDGYRWRKYGQKAVKNSPYPRSYYRCTT---AGCGVKKRVERSSDDP-SVVVTTYEGQHIHQ : 58
CmWRKY3_4 : EDGYNWRKYGQKQVKGSEYPRSYYKCTH---PNCQVKKKVERSLDGQ-ITEII-YKGAHIH- : 56
CmWRKY21_3 : -DDYSWRKYGQKPIKGSPHPRGYYKCSSM--RGCPARKHVERCLEDP-SMLIVTYEGEHNHP : 58
CmWRKY22_1 : -DSWGWRKYGQKPIKGSPYPRSYYRCSSS--KGCSARKQVERSLSDP-GAFVVTYSAEHNH- : 57
CmWRKY41_5 : EDGYSWRKYGQKDILGATFPRSYYRCTFRNTQNCWAIKQVQRS-DEDHSVFDITYRGKHTC- : 60
CmWRKY41_6 : NDGYSWRKYGQKDIHGANFPRCYYRCSHRHERGCLATKQVQRS-DNDPNIFDVTYRGRHTC- : 60
CmWRKY12_1 : DDGYKWRKYGQKVVKNSLHPRSYYRCTH---SNCRVKKRVERLSEDC-RMVITTYEGRHNH- : 57
CmWRKY22_2 : -DIWAWRKYGQKPIKGSPYPRGYYRCSSS--KGCMARKQVERNRSDP-GMFIVTYTAEHNHP : 58
CmWRKY18_3 : KDGYQWRKYGQKVTKGNPSPRAYYKCSFA--PSCPVKKKVQRSVQDS-SYLVATYEGEHNHK : 59
CmWRKY48_3 : DDGYRWRKYGQKAVKNSPYPRSYYRCTT---SQCGVKKRVERSSSDH-SIVITTYEGQHTHQ : 58
CmWRKY22_3 : -DIWAWRKYGQKPIKGSPYPRGYYRCSSS--KGCMARKQVERNRSDP-GMFIVTYTAEHNHP : 58
CmWRKY41_3 : NDGYSWRKYGQKDIHGANFPRCYYRCTHRNVRGCLATKQVQKS-DNDPNIFEVTYRGRHTC- : 60
CmWRKY12_2 : DDGYKWRKYGQKVVKNSLHPRSYYRCTH---SNCRVKKRVERLSEDC-RMVITTYEER---- : 54
CmWRKY2_1 : EDGYNWRKYGQKQVKGSEYPRSYYKCTH---PNCQVKKKVERSNEGH-ITEII-YKGTHNHP : 57
CmWRKY20_2 : DDGYNWRKYGQKHVKGSEFPRSYYKCTH---PNCEVKKLFERSHDGQ-IVDII-YKGTHDHP : 57
CmWRKY70_1 : DDGHAWRKYGQKSIQNAKFPRNYYRCTHKFDQGCQASKQVQRVEEHPPKFRT-TYYGHHTC- : 60
CmWRKY21_4 : -DEYSWRKYGQKPIKGSPHPRGYYKCSSM--RGCPARKHVERCLQQP-SMLIVTYEGEHNHP : 58
CmWRKY42_2 : -DGCQWRKYGQKMAKGNPCPRAYYRCTMA--AGCPVRKQVQRCAEDK-TILITTYEGNHNHP : 58
CmWRKY69_3 : -DSWAWRKYGQKPIKGSPYPRGYYRCSSS--KGCPARKQVERSRVDP-TKLVITYSFDHNHQ : 58
CmWRKY28_2 : EDGYRWRKYGQKAVKNSPYPRSYYRCTS---QKCVVKKRVERSYQDP-SLVITTYEGQHIH- : 57
CmWRKY18_4 : KDGYQWRKYGQKVTRDNPCPRAYFKCSFA--PTCPVKKKVQRSVEDQ-SVLVATYEGEHNHP : 59
CmWRKY23_1 : EDGYRWRKYGQKAVKNSPFPRSYYRCTT---PTCNVKKRVERCSNDP-TILVTTYEGQHTHP : 58
CmWRKY75_2 : DDGYRWRKYGQKAVKNNKFPRSYYRCTH---QGCNVKKQVQRLTRDE-GVVVTTYEGMHTH- : 57
CmWRKY3_5 : -DGYNWRKYGQKQVKGSKYPRSYYKCTH---PCCPVKKKVERSLDGK-IAEIV-YKGEHDHP : 56
CmWRKY33_3 : NDGYNWRKYGQKQVKGSENPRSYYKCTF---PNCPTKKKVERSLDGQ-ITEIV-YKGSHNH- : 56
CmWRKY53_2 : -DGFSWRKYGQKDILGSKFPRGYFRCSHRFTLGCKATKQVQKS-DTDPTIYEVTYKGTHTC- : 59

D WRKYGqK p4 y C3 C Y h h

APPENDIX D

Appendix D.1

The RWC (%), P_N (mmol/m^2.s) under different treatments during the fruit[1]

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
RWC	10 DAP	WW	92.68 ± 3.7	93.59 ± 4.1	93.81 ± 3.5	95.49 ± 3.5
		WW + AMF	93.22 ± 2.2	94.67 ± 3.7	94.72 ± 2.1	96.84 ± 2.3
		DI	75.38 ± 2.8	84.72 ± 2.9	81.29 ± 2.8	87.21 ± 3.2
		DI + AMF	79.59 ± 3.5	90.87 ± 3.4	86.41 ± 3.6	94.28 ± 2.9
RWC	20 DAP	WW	90.32 ± 3.8	91.78 ± 2.2	92.21 ± 2.7	93.49 ± 3.7
		WW + AMF	91.61 ± 4.2	93.86 ± 3.5	94.12 ± 2.2	95.78 ± 3.5
		DI	76.69 ± 3.3	81.29 ± 2.7	80.89 ± 3.5	83.65 ± 2.8
		DI + AMF	80.92 ± 1.8	88.31 ± 2.8	85.91 ± 2.8	91.62 ± 4.1
RWC	30 DAP	WW	83.38 ± 2.8	90.61 ± 3.6	91.18 ± 3.6	92.11 ± 2.7
		WW + AMF	84.05 ± 3.4	92.13 ± 2.1	92.31 ± 2.4	93.85 ± 4.2
		DI	69.29 ± 2.7	80.59 ± 3.2	77.78 ± 3.3	82.26 ± 3.6
		DI + AMF	73.21 ± 3.2	86.87 ± 2.8	82.91 ± 3.1	89.09 ± 2.1
P_N	10 DAP	WW	13.69 ± 0.53	14.43 ± 0.68	15.39 ± 0.72	16.31 ± 0.67
		WW + AMF	13.91 ± 0.47	14.87 ± 0.71	15.82 ± 0.68	16.89 ± 0.73
		DI	8.96 ± 0.36	11.53 ± 0.49	10.39 ± 0.49	13.28 ± 0.41
		DI + AMF	9.72 ± 0.41	13.82 ± 0.56	11.78 ± 0.51	15.97 ± 0.63
P_N	20 DAP	WW	12.61 ± 0.52	13.51 ± 0.53	14.19 ± 0.57	15.71 ± 0.57
		WW + AMF	12.82 ± 0.46	13.89 ± 0.61	14.52 ± 0.63	16.28 ± 0.66
		DI	7.62 ± 0.27	9.32 ± 0.38	9.12 ± 0.35	10.96 ± 0.51
		DI +AMF	8.69 ± 0.32	12.87 ± 0.57	11.28 ± 0.47	15.32 ± 0.68
P_N	30 DAP	WW	10.41 ± 0.48	12.01 ± 0.48	13.89 ± 0.61	14.49 ± 0.58
		WW + AMF	10.59 ± 0.36	12.33 ± 0.52	14.21 ± 0.46	15.02 ± 0.37
		DI	5.78 ± 0.21	8.58 ± 0.27	8.13 ± 0.32	10.47 ± 0.42
		DI +AMF	6.38 ± 0.19	11.52 ± 0.41	9.59 ± 0.37	14.21 ± 0.61

Notes: RWC – relative water content; DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

Appendix D.2

The IAI and SuSy activity [μmol/g (glucose) h] under different treatments during the fruit development stage (mean ± SE)[1]

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
IAI	10 DAP	WW	22.31 ± 0.93	20.19 ± 0.85	25.68 ± 0.86	22.79 ± 0.87
		WW + AMF	22.69 ± 0.87	20.78 ± 0.93	26.31 ± 1.06	23.68 ± 0.95
		DI	23.82 ± 0.63	23.31 ± 1.05	28.09 ± 1.13	26.81 ± 1.13
		DI + AMF	25.38 ± 1.07	26.52 ± 1.13	30.81 ± 1.25	31.12 ± 1.21
	20 DAP	WW	34.38 ± 1.24	31.61 ± 1.23	41.28 ± 1.75	36.69 ± 1.54
		WW + AMF	34.92 ± 1.35	32.75 ± 1.45	42.51 ± 1.36	38.31 ± 1.32
		DI	36.59 ± 1.52	36.09 ± 1.76	44.39 ± 1.97	43.52 ± 1.87
		DI + AMF	38.91 ± 0.95	40.18 ± 1.58	48.58 ± 1.06	49.18 ± 2.13
	30 DAP	WW	27.81 ± 1.17	25.69 ± 1.07	31.89 ± 1.23	28.78 ± 1.07
		WW + AMF	28.26 ± 1.25	26.61 ± 0.97	32.68 ± 1.45	30.09 ± 0.98
		DI	30.19 ± 1.22	28.89 ± 1.14	35.11 ± 1.52	33.92 ± 1.52
		DI + AMF	32.28 ± 1.53	33.12 ± 1.53	38.72 ± 1.21	39.31 ± 1.88
SuSy	10 DAP	WW	15.71 ± 0.54	14.79 ± 0.63	17.21 ± 0.75	16.28 ± 0.62
		WW + AMF	16.02 ± 0.63	15.21 ± 0.71	17.59 ± 0.67	16.91 ± 0.71
		DI	16.68 ± 0.58	16.32 ± 0.58	18.62 ± 0.87	18.22 ± 0.82
		DI + AMF	17.79 ± 0.42	18.48 ± 0.75	20.22 ± 0.46	20.79 ± 0.4
	20 DAP	WW	13.31 ± 0.56	12.68 ± 0.56	15.32 ± 0.67	14.28 ± 0.57
		WW + AMF	13.48 ± 0.61	13.11 ± 0.47	15.68 ± 0.58	14.86 ± 0.64
		DI	14.21 ± 0.65	14.02 ± 0.62	16.64 ± 0.72	16.12 ± 0.72
		DI + AMF	15.19 ± 0.42	15.81 ± 0.73	17.93 ± 0.69	18.41 ± 0.83
	30 DAP	WW	17.41 ± 0.58	16.48 ± 0.68	19.42 ± 0.66	18.18 ± 0.76
		WW + AMF	17.62 ± 0.82	17.02 ± 0.73	19.78 ± 0.75	18.91 ± 0.89
		DI	18.49 ± 0.75	18.21 ± 0.84	20.81 ± 0.93	20.32 ± 0.67
		DI + AMF	19.68 ± 0.45	20.52 ± 0.47	22.59 ± 0.52	23.21 ± 0.56

Notes: IAI – insoluble acid invertase; SuSy – sucrose synthase; DAP – days after pollina- tion; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

Appendix D.3

Sucrose and total sugar content [mg/g (FW)] under different treatments during the fruit development stage (mean ± SE)[1]

Traits	Stages	Treatments	2X	2X/P	3X	3X/P
Sucrose content	10 DAP	WW	4.809 ± 0.21	4.759 ± 0.17	5.331 ± 0.21	5.059 ± 0.21
		WW + AMF	4.871 ± 0.19	4.851 ± 0.16	5.412 ± 0.19	5.182 ± 0.19
		DI	5.102 ± 0.16	5.132 ± 0.21	5.702 ± 0.22	5.552 ± 0.22
		DI + AMF	5.212 ± 0.22	5.416 ± 0.19	5.878 ± 0.17	5.921 ± 0.17
	20 DAP	WW	22.59 ± 0.87	21.31 ± 0.96	25.49 ± 1.05	24.18 ± 0.96
		WW + AMF	22.86 ± 0.69	21.79 ± 0.68	25.97 ± 0.93	24.92 ± 0.87
		DI	24.11 ± 0.93	23.34 ± 0.57	27.51 ± 0.65	26.67 ± 1.06
		DI + AMF	24.39 ± 0.58	24.68 ± 1.06	28.12 ± 1.12	28.47 ± 1.21
	30 DAP	WW	41.79 ± 1.72	40.38 ± 1.35	44.51 ± 1.97	42.81 ± 1.95
		WW + AMF	42.31 ± 1.23	41.31 ± 1.62	45.32 ± 1.76	44.13 ± 1.32
		DI	44.28 ± 1.08	43.81 ± 2.03	47.55 ± 1.35	46.76 ± 1.86
		DI + AMF	45.39 ± 1.78	46.39 ± 1.35	49.16 ± 2.06	50.29 ± 2.13
Total sugar content	10 DAP	WW	51.29 ± 1.83	48.6 ± 1.96	56.41 ± 2.23	53.28 ± 1.98
		WW + AMF	51.85 ± 1.76	50.02 ± 2.07	57.53 ± 1.76	55.21 ± 1.67
		DI	54.33 ± 2.21	52.22 ± 1.84	60.19 ± 2.08	57.86 ± 2.13
		DI + AMF	55.33 ± 2.13	55.52 ± 2.32	61.47 ± 2.45	61.95 ± 2.55
	20 DAP	WW	66.19 ± 2.25	62.91 ± 2.16	71.38 ± 2.96	67.51 ± 2.63
		WW + AMF	67.11 ± 1.57	64.53 ± 1.57	72.87 ± 1.78	69.67 ± 2.34
		DI	70.09 ± 2.84	67.86 ± 2.86	76.12 ± 2.67	74.09 ± 3.12
		DI + AMF	70.92 ± 2.56	71.82 ± 3.22	78.46 ± 3.25	78.82 ± 3.55
	30 DAP	WW	80.48 ± 3.05	76.09 ± 3.19	85.28 ± 3.64	81.12 ± 3.26
		WW + AMF	81.42 ± 2.87	78.03 ± 1.78	86.92 ± 2.05	83.56 ± 2.86
		DI	85.28 ± 3.12	82.12 ± 3.82	91.09 ± 2.79	89.21 ± 2.17
		DI +AMF	86.51 ± 2.09	86.81 ± 4.13	93.58 ± 3.86	94.69 ± 4.12

Notes: DAP – days after pollination; WW – the well-watered; DI – the deficit irrigation; WW + AMF – well-watered and inoculated with arbuscular mycorrhizal fungi; DI + AMF – deficit irrigation and inoculated with arbuscular mycorrhizal fungi; 2X – the diploid watermelon; 2X/P – the diploid watermelon grafted onto the pumpkin; 3X – the triploid watermelon; 3X/P – the triploid watermelon grafted onto the pumpkin

Appendix E

ЗАТВЕРДЖУЮ

Проректор з науково-педагогічної та навчальної роботи,

д.с.н., професор

_____Ігор КОВАЛЕНКО

ДОВІДКА

Про впровадження результатів наукових досліджень у навчальний процес.

Видана Хе Сунтао у тому, що матеріали дисертаційної роботи «Комплексний екологічний, фізіологічний та генетичний аналіз реагування представників роду *Cucurbita* на сольовий стрес», які опубліковані в статтях:

Peng-Ming Yang & **Song-Tao He** (2022). The effects of arbuscular mycorrhizal fungi and deficit irrigation on the yield and sugar content of watermelons (*Citrullus lanatus*), Horticultural Science (Prague), 1–9. https://doi.org/10.17221/108/2021-HORTSCI;

Хе Сунтао (2022). Фізіолого-біохімічні аспекти реагування рослин на засолення ґрунту (оглядова). Вісник Сумського національного аграрного університету. Серія: Агрономія і біологія, 50 (4), 62–68. https://doi.org/10.32845/agrobio.2022.4.9;

Хе Сунтао (2023). Щеплення у системі заходів із вирощування рослин родини *Cucurbitaceae*. Вісник Сумського національного аграрного університету. Серія «Агрономія і біологія», 1 (51). С.129–136. DOI https://doi.org/10.32782/agrobio.2023.1.15;

He Songtao, Skliar V.G. & Zhou Junguo (2019). Effects of different concentrations of salt on pumpkin seedlings. Матеріали науково-практичної конференції викладачів, аспірантів та студентів Сумського НАУ (17–20 квітня 2019 р.), 29;

He Songtao, Zhou Junguo & Skliar V.G. (2019). The problem of soil salinization and the role of genetic engineering in increasing the salt tolerance of

plants. Матеріали Міжнародної науково-практичної конференції, присвяченої 90-річчю з дня народження доктора сільськогосподарських наук, професора Гончарова Миколи Дем'яновича (24–25 травня 2019 р.), 13;

включені до навчальних програм (силабусів) дисциплін «Екологічна фізіологія рослин», «Агроекологія», «Біологія» та використовуються в навчальному процесі підготовки здобувачів вищої освіти спеціальності 101 «Екологія».

Довідка видана для подання до спеціалізованої вченої ради.

Завідувач кафедри
екології та ботаніки Вікторія СКЛЯР