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Thesis

CREATION OF THE SOURCE MATERIAL OF WINTER WHEAT RESISTANT TO CADMIUM ACCUMULATION

Specialty 201 "Agronomy".

20 Agricultiral Sciences and Food production

for a Doctor Philosophy Degree (PhD)

The dissertation contains the results of own research. The use of ideas, results and texts of other authors are linked to the corresponding source

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ANNOTATION

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Wheat is an archeophyte crop that has a sown area of over 200 million hectares and a gross harvest is approximately 800 million tons. Wheat cultivation and consumption are prevalent in the cultures of the majority of the world's peoples. In the absence of reserves to expand sown areas, the main direction of stable wheat consumption is to increase yields and increase the share of crops suitable for the production of environmentally friendly food. Realization of this direction is possible due to developing and introduction in production of varieties with the controlled level of accumulation of separate substances and elements. It needs the formation of theoretical base of inheritance feature and genetic control of qualitative breeding traits.

Crops with appropriate quality indicators are used in the production of food and animal feed. This is especially true for heavy metals content that can migrate up the food chain. Wheat is a crop species with a high level of cadmium assimilation and accumulation in the seeds - one of the most toxic heavy metals. The urgent task of modern science is to expand theoretical research and solve practical problems in order to create varieties with a controlled level of cadmium accumulation.

The dissertation work provides a theoretical foundation and practical solution

to the issues of creating source material of winter wheat with the low Cd - uptake for breeding purpose and establishing molecular - genetic basis of plant cadmium accumulation ability.

There were studied collection of varieties representing 7 major breeding centers of wheat in Ukraine. As a result of studying the collection samples of wheat from different institution - originators, samples with valuable breeding characteristics were identified. Growth parameters (height, stem weight, leaf area) were analyzed in wheat varieties from the collection. These traits were related to productivity parameters such as 1000 seed weight, grain weight per ear and yield.

Genetic differences in the control mechanisms of resistance trait to cadmium accumulation have been established. The range of variation of the cadmium content trait in plants and grains of winter wheat varieties was determined to be 0.91 - 2.02 and 0.06 - 2.56 mg / kg, respectively. Varieties had minimum indicators of cadmium content in grains: Oktava odes'ka, Svitanok myronivskyi, Melody odes'ka, Kubok, Shchedra nyva. The maximum level of cadmium content was noted in varieties of Rozkvit, Sich, Kantata odesk'a, Duma odesk'a.

A group of varieties with the lowest and high cadmium content was formed based on cadmium content values and plant productivity parameters.

In groups of varieties with different cadmium content, differences in the structure of correlations between the main selection - controlled parameters were established. It was determined that the group of "low cadmium varieties" is characterized by the presence of direct correlations between the cadmium content and parameters of vegetative development of plants, namely plant height (r=0.54)

and stem weight (r=0.88). In addition, a reliable negative correlation was noted with the indicators of the seed mass per ear (r= - 0.60) and 1000 seed weight (r= - 0.74). Thus, the variation coefficient of yield in the group of varieties with a low level of cadmium accumulation was 9.24% versus 6.14% for the group with a high ability to accumulate this metal.

In the group of varieties with a high content of cadmium, a reliable level of correlation of the cadmium content trait with the LAR indicator (r= - 0.68) and the absence of statistically significant relationships with plant productivity indicators was noted. Significant differences in the correlation structure of cadmium content trait in seeds from high and low cadmium content variety groups indicates a difference in the mechanisms of genetic control of this characteristic.

In the conditions of the field experiment, 40 intervarietal hybrids were obtained and studied. The distribution of inheritance frequencies of resistance to cadmium accumulation trait in F_1 was calculated. After crossing low - cadmium varieties, the frequency of inheritance according to the type of heterosis was 12%, incomplete positive dominance - 8%, intermediate inheritance - 16%, and according to the type of depression and incomplete negative dominance - 44%. Inheritance according to the type of heterosis was noted in such combinations as Melody odes'ka x Ovidyi, Kubok x Svitanok myronivskyi and Shchedra nyva x Okhtyrchanka juvileyna.

It was established that a characteristic feature of hybrids with minimal cadmium content in seeds was inverse and statistically significant correlations between the values of cadmium content and indicators of the seed weight per ear

(r= - 0.70), 1000 seed weight (r= - 0.78) and the indicator of estimated yield (r = - 0.74). In the group of hybrids with a high level of cadmium concentration in seeds, a reliable level of correlation between the values of this indicator and the controlled traits was not found.

Six samples were allocated for perspective breeding work: 19/1; 19/13; 19/26; 19/40; 19/33; 19/39, obtained in combinations of Melody odesk'a x Svitanok myronivskyi; Melody odesk'a x Shchedra nyva; Kubok x Svitanok myronivskyi; Shchedra nyva x Kubok; Zorepad x Okhtyrchanka juvileyna; Shchedra nyva x Okhtyrchanka juvileyna. The samples exceed the conventional standart in terms of cadmium content in seeds and one of the indicators of yield structure.

Molecular biology approach was used to study the molecular mechanism of cadmium resistance in wheat. The role of individual genes in the control of processes and mechanisms of cadmium accumulation in winter wheat plants was determined. Specific role of *TaSFT2L* in the control of this element uptake was established. *TaSFT2L* - novel *GOT/SFT2s* member termed *SFT2L* was functionally characterized. The gene *TaSFT2L* plays an important role in the transport of Cd and is mainly expressed in roots and induced by this metal. Cd toxicity caused rapid Cd - induced up - regulation of *TaSFT2L* in roots, the main site of Cd toxicity and input in plants. This fact suggests a specific role of *TaSFT2L* in Cd plant tolerance.

The TaSFT2L sequence was found to be highly conserved and exhibited a high homology with other plant species. The TaSFT2L - GFP fusion protein was determined to be localized in the cytomembrane of wheat cell protoplasts. It was studied TaSFT2L involved in tolerance to the cadmium in wheat and usually targets

cell membranes. Vacuolar separation of Cd - induced TaSFT2L silencing in plants is a Cd tolerance mechanism in wheat.

TaSFT2L was determined as negative regulator of Cd uptake. It was determined *TaSFT2L* gene expression in wheat suppresses the accumulation of Cd. RNAi/OE wheat lines expressing *TaSFT2L* were prepared and they were exposed to Cd stress. RNA interference (RNAi) of *TaSFT2L* resulted in improved plant height, biomass, and chlorophyll accumulation. Cd contents in the grain decreased by 29.40 - 74.95% and the proportion of Cd translocated from the roots to grains decreased by nearly 68% in RNAi lines of the *TaSFT2L*.

The overexpression of *TaSFT2L* due to cadmium toxicity resulted in compromised growth response, with higher cadmium accumulation in wheat tissues. The results proved that *TaSFT2L* was a key gene regulating Cd translocation in wheat. It was reaveld that silencing the functional gene *TaSFT2L* to form transgenic wheat can inhibit Cd accumulation in wheat grains.

The *TaSFT2L* gene from wheat was functionally identified, and it demonstrated that preventing Cd from building up in roots can prevent Cd from transferring to grains. *TaSFT2L* RNAi lines of wheat exhibited decreased Cd levels in roots by 28.13 - 36.04% and reduced proportions of Cd transferred from roots to grains by about 68%.

TaSFT2L silencing is potentially useful since it can help to create new genotypes in breeding programs with low Cd accumulation in wheat grains.

An original sourse material was created, which is being researched and refined in scientific programs at Sumy National Agrarian University, the Institute of

Agriculture of North-East of Ukraine and Henan Institute of Science and Technology (Xinxiang, China).

Based on the results of the research, a working collection of winter wheat samples with a low level of cadmium accumulation was transferred to the laboratory of selection and seed production of the Institute of Agriculture of North - East of Ukraine; materials of study are included in educational programs on disciplines of educational level of Bachelor of 201 Agronomy and 204 Ecology specialties at Sumy NAU.

The *SFT2L* gene has been patented in China (application 20039041 dated October 29, 2020). (Appendex E)

Key words: *winter wheat (Triticum aestivum L.), cadmium, collection, variety, hybridization, crossing, source material, growth and development, morphological indicators of plants, seeds, correlation, breeding, valuable traits, variability, genotype, productivity, yeild structure, yield, tolerance, Cd-accumulation, Cd resistance, molecular mechanism, genes, breeding value.*

АНОТАЦІЯ

У Люлю. Створення вихідного матеріалу озимої пшениці, стійкого до накопичення кадмію. - Рукопис дисертації на здобуття наукового ступеня доктора філософії (PhD): спеціальність 201 «Агрономія». – Сумський національний аграрний університет, Суми, 2022

Пшениця — культура-археофіт, посівна площа якої становить понад 200 млн га, а валовий збір - біля 800 млн т. Вирощування та споживання пшениці поширено в культурах більшості народів світу. За відсутності резервів розширення посівних площ основним напрямком стабільного споживання є підвищення врожайності та збільшення частки культур, придатних для виробництва екологічно чистих продуктів харчування. Реалізація цього напряму можлива завдяки розробці та впровадженню у виробництво сортів із контрольованим рівнем накопичення окремих речовин і елементів. Це потребує формування теоретичних основ успадкування ознак і генетичного контролю якісних та селекційно-цінних параметрів.

Культури з відповідними показниками якості використовують у виробництві харчових продуктів і кормів для тварин. Особливо це стосується вмісту важких металів, які можуть мігрувати харчовим ланцюгом.

Пшениця – вид культури з високим рівнем засвоєння та накопичення в насінні кадмію – одного з найбільш токсичних важких металів. Актуальним завданням сучасної науки є розширення теоретичних досліджень і вирішення практичних завдань з метою створення сортів з контрольованим рівнем накопичення кадмію.

У дисертаційній роботі представлено теоретичне обґрунтування та практичне вирішення питань створення вихідного матеріалу пшениці озимої з низьким вмістом Cd - для селекційних цілей та встановлення молекулярно-генетичних основ здатності рослин пшениці озимої до накопичення цього металу.

Досліджено колекцію сортів, які представляють 7 основних селекційних центрів пшениці озимої в Україні. У результаті вивчення колекційних зразків пшениці різних установ-оригінаторів виявлено сорти з цінними селекційними властивостями. У колекційних сортів пшениці проведено аналіз показників росту (висота, маса стебла, площа листків). Ці ознаки були пов’язані з такими параметрами продуктивності, як маса 1000 насінин, маса зерна в колосі та врожайність.

Встановлено генетичні відмінності в механізмах контролю ознаки стійкості до накопичення кадмію. В сортів пшениці озимої визначено діапазон варіювання ознаки вмісту кадмію в вегетативних органах та зерні - 0,91 – 2,02 мг/кг та 0,06 – 2,56 мг/кг відповідно. Мінімальні показники вмісту металу в зерні мали сорти: Октава одеська, Світанок миронівський, Мелодія одеська, Кубок, Щедра нива. Максимальний рівень вмісту кадмію виявлено в сортів Розквіт, Січ, Кантата одеська, Дума одеська.

За показниками вмісту кадмію та показниками продуктивності рослин сформовано групи сортів з найнижчим та високим вмістом кадмію. У групах сортів з різним вмістом кадмію встановлено відмінності в структурі кореляційних зв’язків між основними селекційно–контрольованими

показниками.

Визначено, що група “малокадмієві сорти” характеризується наявністю прямих кореляційних зв’язків між вмістом кадмію та параметрами вегетативного розвитку рослин, а саме: висотою рослини (r=0,54) та масою стебла (r=0,88). Крім того, відзначено достовірну негативну кореляцію з показниками маси насіння в колосі (r= – 0,60) та маси 1000 насіння (r= – 0,74). Коефіцієнт варіації показника врожайності в групі сортів із низьким рівнем накопичення кадмію становив 9,24 % проти 6,14 % у групі з високою здатністю до накопичення цього металу.

У групі сортів з високим вмістом кадмію відзначено достовірний рівень кореляції ознаки “вміст кадмію” з показником LAR (r= – 0,68) та відсутність статистично значущих зв’язків з показниками продуктивності рослин. Суттєві відмінності кореляційної структури ознаки вмісту кадмію в насінні високо- та низькокадмієвих сортогруп свідчать про різницю в механізмах генетичного контролю цієї ознаки.

В умовах польового досліду було отримано та досліджено 40 міжсортових гібридів. Розраховано розподіл частот успадкування ознаки стійкості до накопичення кадмію у F_1.

Після схрещування низькокадмієвих сортів частота успадкування за типом гетерозису становила 12%, за типом неповного позитивного домінування – 8%, проміжного успадкування – 16%, а за типом депресії та неповного негативного домінування – 44%. Успадкування за типом гетерозису відзначено у таких комбінаціях, як Мелодія одеська х Овідій, Кубок х

Світанок миронівський та Щедра нива х Охтирчанка ювілейна.

Встановлено, що характерною ознакою гібридів з мінімальним вмістом кадмію в насінні є зворотні та статистично значущі кореляційні зв'язки між значеннями вмісту кадмію та показниками маси насіння в колосі (r= – 0,70), маси 1000 насінин (r= – 0,78) та показником розрахункової врожайності (r = -0,74).

У групі гібридів із високим рівнем концентрації кадмію в насінні достовірного рівня кореляції між значеннями цього показника та контрольованими ознаками не виявлено.

Для перспективної селекційної роботи виділено 6 зразків: 19/1; 19/13; 19/26; 19/40; 19/33; 19/39, отриманих в схрещуваннях Мелодія одеська х Світанок миронівський; Мелодія одеська х Щедра нива; Кубок х Світанок миронівський; Щедра нива х Кубок; Зорепад х Охтирчанка ювілейна; Щедра нива х Охтирчанка ювілейна. Зразки перевищують умовний стандарт за одним із показників структури врожаю та характеризуються нижчим вмістом кадмію в насінні.

Для вивчення молекулярного механізму стійкості пшениці до кадмію використовували методи молекулярної біології. Визначено роль окремих генів у контролі процесів і механізмів накопичення кадмію в рослинах озимої пшениці.

Встановлено роль гену *TaSFT2L* у контролі поглинання цього елемента. *TaSFT2L* - новий член родини *GOT/SFT2s*, (назва *SFT2L*). Ген *TaSFT2L* відіграє важливу роль у транспорті Cd і в основному експресується в коренях та

індукується цим металом. Висока концентрація кадмію викликала швидку, (індуковану металом) регуляцію *TaSFT2L* у коренях - головному місці надходження елементу в рослини.

Було виявлено, що послідовність TaSFT2L є висококонсервативною та демонструє високу гомологію з іншими видами рослин. Визначено, що білок TaSFT2L - GFP локалізований у цитомембрані протопластів клітин пшениці. Досліджено, що саме TaSFT2L бере участь у формуванні толерантності пшениці до кадмію та зазвичай діє на клітинні мембрани. Вакуолярна сепарація сайлесингу TaSFT2L, викликаного Cd є механізмом толерантності до металу в рослин пшениці.

Ген *TaSFT2L* визначено як негативний регулятор поглинання Cd. Встановлено, що експресія гена *TaSFT2L* у пшениці пригнічує накопичення металу. Було показано, що блокування накопичення кадмію в коренях може запобігти транспорту кадмію до насіння.

Отримано лінії RNAi/OE, що експресують *TaSFT2L*, вони були проаналізовані на стресостійкість до Cd. РНК-інтерференція (RNAi) *TaSFT2L* призвела до покращення таких параметрів, як висота рослин, біомаса та накопичення хлорофілу. Вміст кадмію в зерні зменшився на 29,40-74,95%, а частка кадмію, перенесеного з коренів у насіння, зменшилася майже на 68% у лініях RNAi *TaSFT2L*.

Надмірна експресія *TaSFT2L* через токсичність кадмію призвела до порушення реакції росту з більшим накопиченням металу в тканинах пшениці. Результати довели, що *TaSFT2L* є ключовим геном, який регулює

транслокацію Cd у пшениці. Виявлено, що сайлесинг функціонального гена *TaSFT2L* при створенні трансгенної пшениці може пригнічувати накопичення металу в зерні.

Сайлесинг *TaSFT2L* є потенційно корисним в майбутніх селекційних програмах зі створення нових генотипів з низьким накопиченням кадмію в зерні пшениці озимої.

Створено оригінальний вихідний матеріал, який досліджується та удосконалюється в наукових програмах Сумського національного аграрного університету, Інституту сільського господарства Північного Сходу НААН України та Хенанського науково-технологічного інституту (Сіньсян, Китай).

За результатами досліджень робочу колекцію зразків озимої пшениці з низьким рівнем накопичення кадмію передано до лабораторії селекції та насінництва Інституту сільського господарства Північного Сходу України; навчальні матеріали входять до навчальних програм з дисциплін освітнього рівня бакалавра спеціальностей 201 Агрономія та 204 Екологія Сумського НАУ. Ген *SFT2L* запатентовано в Китаї (заявка 20039041 від 29 жовтня 2020 року). (Додаток Е)

Ключові слова: *пшениця озима (Triticum aestivum L.), кадмій, колекція, сорт, гібридизація, схрещування, вихідний матеріал, ріст і розвиток, морфологічні показники рослин, насіння, кореляція, селекція, цінні ознаки, мінливість, генотип, продуктивність, структура врожаю, урожайність, толерантність, акумуляція кадмію, Cd-стійкість, молекулярний механізм, гени, селекційна цінність.*

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Abstracts of conferences

8. **Wu Liuliu,** G. A. Zhatova, Li Cheng Wei. Cloning and analysis of cadmium - stress related genes in wheat (*Triticum aestivum*). 6th International Symposium on Genomics and Crop Genetic Improvement - Molecular Breeding Conference. 2019, Wuhan: Huazhong Agricultural University. 2019, P.41.

9. **Wu Liuliu,** Zhatova H. O. Basis for the breeding of wheat varieties with Cd - low content. Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya», 2019, P. 82

10. **Wu Liuliu,** Li Chengwei, H. Zhatova. Current status of wheat cadmium accumulation in China. Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya». 2020, P.43

11. **Wu Liuliu,** Zhatova H. O. Functional characterization of a new vesicle transport protein for reducing cadmium concentration in wheat crop. Сумський національний аграрний університет, Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya». 2021, P.111

CONTENT

ABBREVIATIONS

Cd – cadmium.

GOT1 / $SFT2_S$ – the vesicle transporter family.

VIGS –virus induced gene silencing.

ABC – the ATP - Binding Cassette Transporter gene family.

ZIP – the Zrt/ IRt protein.

CDF – the cation diffusion facilitator.

LCT – the low - affinity transporter.

ROS – reactive oxygen species.

NRAMP – natural resistance - associated macrophage protein.

WT – wild - type.

MS – Murashige - Skoog medium.

NCBI BLAST – the basic local alignment search tool of National Center for Biotechnology Information Search database.

Kan – Kanamycin.

ICP - MS, –inductively coupled plasma mass spectrometry.

OPT – oligopeptide transporter.

YNB – yeast nitrogen base without amino acids medium.

PMSF – phenylmethylsulfonyl fluoride.

BSMV – barley stripe mosaic virus.

PDS – phytoene desaturase.

CDS – the coding sequence.

TTC – the triphenyl tetrazolium chloride.

FDA - PI – fluorescein diacetate and propidium iodide.

H_2O_2 – hydrogen peroxide;

$O^{\bullet -}$, superoxide radicals.

DCF - DA – 2 - 7′ - dichlorofluorescein diacetate.

DHE – dihydroethidium.

TEM – the transmission electron microscopy.

RNAi – RNA interference.

OE – overexpression.

INTRODUCTION

Wheat is an archeophyte crop with a sown area of over 200 million hectares, and a gross harvest is approximately 800 million tons. Wheat cultivation and consumption are prevalent in the cultures of the majority of the world's peoples.

A significant segment of modern society is associated with the cultivation and processing of crops, production and logistics focused on these processes by Agricultural machinery, fertilizers and plant protection products.

In the absence of reserves to expand sown areas, the main direction of stable wheat consumption is to increase yields and increase the share of crops suitable for the production of environmentally friendly food.

Realization of this direction is possible due to developing and introduction in production of varieties with the controlled level of accumulation of separate substances and elements. It needs the formation of theoretical base of inheritance feature and genetic control of qualitative selection traits.

Actuality of theme. The production of food and animal feed is based on the use of crops with appropriate quality indicators. This is especially true for the content of heavy metals capable to migrate along the trophic chain. Wheat belongs to the crop species with a high level of assimilation and accumulation in the seeds cadmium - one of the most toxic heavy metals. The urgent task of modern science is to expand theoretical research and solve practical problems to create varieties with a controlled level of cadmium accumulation.

Connection of work with scientific programs, plans, themes. The research was carried out in accordance with the thematic plans of research works of Sumy National Agrarian University in the framework of the topic «Creating the source material of cereals and oilseeds resistant to the accumulation of heavy metals» (state registration number 0119U101581, 2019 - 2023).

The purpose and objectives of the study. The aim of the research was to increase the efficiency of winter wheat by creating a source material with a controlled level of cadmium accumulation in the crop. The goal was to solve the following tasks:

1. To study the gene pool of domestic varieties of winter wheat on the basis of resistance to cadmium accumulation;

2. To estimate the relationship of the sign of resistance to cadmium accumulation with a set of economically valuable traits;

3. To carry out hybridization of selected samples and evaluation of created hybrids on a set of economically valuable traits and resistance to Cd accumulation;

4. To determine the role of individual genes in the control of processes and mechanisms of cadmium accumulation in winter wheat plants.

Object of study. Regularities of manifestation of economic, morphological and genetic traits in winter wheat varieties and establishment of selection value on their basis.

Subject of study. Breeding value and biological features of the source material

of winter wheat by the main characteristics and ability to accumulate cadmium

Research methods. General scientific methods: analysis, induction, deduction, synthesis; field methods - phenological observations of the collection of winter wheat varieties to determine the selection value of samples; special breeding methods – intravarietal crooss - breeding, determination of biometric parameters of plant growth and development, in particular measuring and weighing, structural analysis; statistical methods - to generalize and determine the reliability of certain experimental results (variation, correlation, dispersion, cluster).

The scientific novelty of the obtained results lies in solving an important scientific problem of developing and evaluating the source material of winter wheat with a controlled level of cadmium accumulation.

For the first time a working collection was formed and valuable source material with minimum and maximum levels of cadmium accumulation were identified. Genetic differences in the mechanisms of control of the trait resistance to cadmium accumulation have been established. The original initial material of winter wheat varieties with a low level of cadmium accumulation has been created. The role of *TaSFT2L* in the control of cadmium accumulation in winter wheat plants was established.

It was improved the scheme of evaluation of breeding material of winter wheat on the traits of resistance to cadmium accumulation.

The issue of quality crop improving of self-pollinating species by controlling valuable breeding traits has been further developed.

The practical significance of the results. Based on the results of the research, an original breeding material with a low level of cadmium accumulation was created, which is being researched and refined in scientific programs at Sumy National Agrarian University, the Institute of Agriculture of North - East of Ukraine and Henan Institute of Science and Technology (Xinxiang, China).

Materials of study are included in educational programs on disciplines of educational level of Bachelor of 201 Agronomy and 204 Ecology specialties at Sumy NAU.

The special role of *TaSFT2L* in the control of processes and mechanisms of cadmium accumulation in winter wheat plants was determined. The *TaSFT2L* sequence was found to be highly conserved and show high homology with other plant species. It was revealed that *TaSFT2L* was a negative regulator of Cd stress Functional identification of the *TaSFT2L* gene from wheat proved that reducing Cd accumulation in roots can inhibit Cd transfer to grains. The gene could be used as a cadmium transporter and play an important role in Cd detoxification of wheat. *TaSFT2L* silencing gene from wheat is potentially useful since it can help to generate genetically modified genotype materials with low Cd accumulation in wheat grains. The gene has been patented in China.

The personal contribution of the applicant is to plan and carry out research, summarize scientific data of references (literature) on the topic of the dissertation, to do analysis of experimental data, to form conclusions and proposals for selection, to prepare and write of scientific papers. Scientific articles have been published

both independently and in co –authorship as well.

Approbation of dissertation results. The results of the research were published and discussed at “The 6-th International Symposium on Genomics and Crop Genetic Improvement - molecular Breeding” conference (2019), at “Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya»” international ones (2019-2021). The main items, research results and conclusions of the work during 2019 - 2022 were presented and discussed at the meetings of the Department of Agrotechnology and Soil Science of Sumy National Agrarian University.

Publications. Based on the results of the research, 4 articles were published in professional journals, two - in journals indexed in the Scopus database, one - in journal of ES and in the proceedings of four conferences.

The structure and scope of the dissertation. The dissertation structure contains an annotation, a list of symbols, introduction, five chapters, conclusions, proposals for breeding practice, a list of references, appendexes.

CHAPTER 1

WAYS TO REDUCE CADMIUM ACCUMULATION BY AGRICULTURAL PLANTS (LITERATURE REVIEW)

Winter wheat is the leading crop in the world and ensures human needs for food. Winter wheat collections are studied in leading breeding institutions in Ukraine and China, which makes it possible to identify sources and donors of the necessary breeding characteristics and involve them in hybridization. A systematic and successful hybridization program requires a thorough understanding of the genotype and genetic architecture of the crop yield and other important economic traits**.**

One of the topical areas of breeding work is obtaining source material with a low ability to accumulate heavy metals, in particular cadmium. It is imperative to minimize Cd contamination in winter wheat seeds. In many countries, solutions are being developed to reduce the content of cadmium in wheat grains.

1.1. Current situation and harm of cadmium pollution

Heavy metal soil pollution has been a serious problem to crop production all around the world [1 - 6]. Heavy metals in excess or harmful concentrations in the soil are absorbed by plant roots and cause long - term heavy metal stress in the plant. Through numerous defense and regulatory mechanisms, plants can lessen or avoid heavy metal toxicity, enabling them to flourish fully even in environments with high concentrations of heavy metals. The first detoxification tolerance is the name of this defense mechanism. Controlling the accumulation of heavy metals in soil and preventing heavy metal stress on plants are difficult tasks, thus it's important to

master the detoxification and defense mechanisms of plants against heavy metals as well as their absorption, transport, and accumulation processes [7, 8].

Internal tolerance and external rejection are the two categories into which plant tolerance and detoxification processes fall. The internal tolerance mechanism in plants is the complexation and chelation of some substances with heavy metals [9]. Limiting heavy metals in some specific tissue parts of plants can reduce the damaging influence of heavy metals and alleviate their toxic effects on plant organisms. External rejection occurs when heavy metal ions are prevented from entering plant cells or when excessive heavy metals are expelled to avoid accumulation. These detoxification mechanisms are mutually reinforcing rather than independent [9]. Cadmium (Cd) is one of the most toxic heavy metals to plants and is considered one of the most toxic elements released into environments even at very low concentrations because it is non - essential nutrient element for plants [10,11,12]. Cd content in agricultural soil has increased due to the development of industry and agriculture [11,13,14, 15,16]. Cadmium enters the soil environment via phosphate fertilizer, animal manures, waste water and garbage from the metal and cement industries, and Cd - contaminated sludge and fertilizer [17 - 21].

Cd accumulation to phytotoxic levels in plants may result in growth disturbances and yield loss. Cadmium is characterized by its high mobility in soil. Its concentration above the critical level can hinder plant metabolism and cause cell death by interfering with various biochemical and physiological processes such as decreased the Intracellular space and chloroplasts, stimulation of reactive oxygen species (ROS), leading to cell membrane damage and destruction of cell organelles

[22 - 28].

The main source of Cd entry into humans via the trophic chain is plants growing in Cd - contaminated soil and Cd - contaminated food. As a result, this metal may be an element with a high residue, which is difficult to degrade and easy to accumulate, posing a serious threat to human and animal health [1, 3, 29, 30, 31].

Plants have various mechanisms to protect Cd uptake and accumulation in seeds including exclusion at root level, Cd compartmentalization, and the formation of stress proteins [32 - 38]. Cadmium uptake, translocation and toxicity in plants is also affected by interaction with othermineral micronutrients such as Si, Pb, Cu and Zn [39 - 44].

1.2. Detoxification mechanism of heavy metals in plants

The root system is the first barrier for cadmium to enter the plant, and it is the most important part in reducing its toxic effect. When the root system is under cadmium stress, it secretes some organic acids and sugars [45]. Plant roots can form soluble complexs with cadmium and other heavy metal ions, which reduces the efficiency and mobility of heavy metal ions, inhibits heavy metal ion transport, and thus reduces plant absorption of these elements.

Studies have shown that under heavy metal stress, plant roots change the form and availability of heavy metals in rhizosphere by regulating the composition of organic acids with low molecular weight, so that plants can adapt to the external environment [46]. Other studies have shown that maize root tips can secrete viscous substances with strong affinity, which reduces the mobility of metal ions, and thus

most metal ions are retained outside the roots [47].

Studies have shown that plants can automatically adjust the pH gradient distribution in the rhizosphere environment when exposed to Cd, and Cd deposition around the root system, reducing Cd entry into the plant organism. The detoxification of plant root exudates plays a connecting role in coping with heavy metal stress.

The plant cell wall is the necessary component and primary site for Cd absorption in plant roots [48]. It plays an important role in plant tolerance and detoxification. Negatively charged binding sites in the cell wall connect positively charged metal ions [9], adsorb or complex heavy metal ions, and prevent cadmium and other heavy metals from entering the cell interior.

Plant cell walls can be modified to improve their ability to accumulate and detoxify heavy metals. Heavy metal ions have been found to enter cell protoplasts in cell walls to protect and reduce the damage caused by heavy metal Cd to plant cells [49].

Under Cd and other heavy metals stress, the activity of pectin methyl ester in plant cell wall increased, and the pectin content grew and rearranged in space, significantly improving plant cell wall absorption and accumulation capacity to heavy metals [50, 51].

Other researchers have found that increasing the contents of cysteine protein and hemicell ulose in root cell wall can significantly inprove tplant cell wall adsorption and fixation to heavy metal ions [52].

In conclusion, the fixing and adsorption of cell wall can restrict the entry of

heavy metal ions into the cytoplasm. Cell wall is engaged in the transport mechanism of heavy metal ions in plant cells. Additionally, the plasma membrane serves as a vital barrier against the entry of heavy metal ions and a natural barrier for material exchange in plant cells. The transmembrane transport of cadmium ions in cells is impacted by the plasma membrane's surface's strong electronegativity, which can adsorb positively charged cadmium ions on its surface.

Studies have demonstrated that plasma membrane transporters are essential for the selective transport of metal ions and can transport metal ions to the extracellular level [50, 53]. The ABC membrane transporter is the main representative of membrane transporter. The transport of heavy metal Cd to vacuoles via the plasma membrane - located metal - excreting protein OsPDR9 has been revealed to be closely associated to the detoxification of heavy metals [54].

Heavy metal transporters, such as AtPDR8, AtHMA2 and AtHMA4, are involved in excreting heavy metal Cd from the cytoplasm and transported to the cell wall to precipitate heavy metals outside the cell membrane [55 - 60]. The capacity of plant cells to isolate heavy metal ions within vacuoles is known as vacuole compartmentalization. Cadmium accumulates in vacuoles when it penetrates the plant cell wall and reaches the protoplast through the plasma membrane [61, 62].

The vacuole contains a lot of organic acids, which might combine with the hazardous heavy metal cadmium to lessen its toxicity [63, 64]. The ability of poplar seedlings to recover from Cd - contaminated soil may be enhanced by the expression of the vacuolar transporter gene *ScYCF1*. Chelating peptide (PCs), which was developed for plant study, can interact with heavy metals like cadmium

to create a complex, enter the vacuole, and combine to reduce the toxicity of high molecular weight chemicals.

Heavy metals are transferred in chelate form to the vacuole via the membrane transporters HMT1, HMT2, which also lessen the toxicity of heavy metals to plants [65 – 67]. It is believed that vacuolar compartmentalization works well to reduce cadmium toxicity. To change the mobility and utilization of heavy metal ions and lessen their toxicity to plants, heavy metal ions are chelated in plants primarily by promoting the formation of metal ligands in the form of metal - organic ligands [68 - 70]. The two most extensively studied proteins that chelate heavy metal ions are metallothionein (MT) and phytochelin (PC) [71 - 72].

Some heavy metals can transform into non - toxic or less toxic combination forms after passing through the cell wall and membrane and entering the cytoplasm, which lessens the toxic effects of heavy metals on plants. These complex stable chelates are formed with organic acids, proteins, and other substances. Rice plants are less hazardous to cadmium when they are exposed to external cadmium stress because PCs, MT, and cadmium mix to generate non - toxic compounds that lower the concentration of intracellular free heavy metal ions [73 - 74].

The antioxidant system in plants is a widely available detoxification strategy. The antioxidant system in plants can protect cells from oxidative stress by scavenging free radicals produced by heavy metal stress [75 - 78]. Studies have shown that scavenging free radicals produced by heavy metal stress can improve tolerance of plants to heavy metals [79]. Under heavy metal stress, rice produces a variety of antioxidant defense mechanisms that scavenge oxygen free radicals and

shield plant cells from being damaged [80 - 82].

It was found that under copper and cadmium stress, wheat leaves create substantial amounts of GSH in order to effectively remove reactive oxygen species and free radicals and prevent membrane lipid peroxidation, suggesting that GSH plays a major role in leaf detoxification. To comprehend the physiological mechanisms of plant stress tolerance and heavy metal detoxification, it is therefore crucial to investigate the antioxidant system. Heavy metal transporters play a crucial function in tolerance mechanisms such organic ligand chelation and vacuolar compartmentation as well as heavy metal absorption and transport in plants.

Heavy metal transporters can transport heavy metal ions out of the cytoplasm or localize them into specific organelles. They can also move heavy metals from non - plastids or organelles into the cytoplasm for detoxification of heavy metals. ATP - binding cassette transporters (ABC transporters) are widely distributed in prokaryotes and eukaryotes. They mainly bind and hydrolyze ATP to release energy to achieve substrate transport across membranes. The ABC transporter gene enhances plants' resistance to heavy metals by mediating the movement of heavy metal ions like cadmium, lead, and aluminum. [83].

Many researchers have found that heavy metals can be transported to vacuoles by heavy metal excretion proteins connected to vacuolar compartmentalization in plants. Extra Zn^{2+} can be transferred via the transporters AHMTP1 and AMTP1 to remove extra zinc from the cell. Cellular Zn^{2+} damage is lessened by compartmentalization [84 – 86]. The heavy metal absorption proteins AtNRAMP1,

AtNRAMP3, and AtNRAMP4 have been demonstrated in other investigations to be capable of moving heavy metal ions like Cd from vacuoles to the cytoplasm [87, 88]. OsPDR9's ability to transport cadmium to vacuoles has been established [89]. Additional research has demonstrated that the proteins Athma1 and Athma6 can move extra Zn and Cu from the cytoplasm to the chloroplast [90 – 91]. Proteins AtHMA7 and AtHMA8 can transport excessive Cu from cytoplasm and chloroplast matrix to Golgi apparatus and thylakoid [92, 93]. The Zinc - iron transporter (ZIP) family (such as IRT1, Osirt1, Osirt2) is involved in the absorption and chelation of Cd and other heavy metals in plants [94 - 96]. Thus, for different kinds of heavy metal transport protein, under the heavy metal stress, transportation way and the way of tolerance is differ. of different heavy metals in the same plant, detoxification of heavy metals by ion transporters, and the possibility of joint coordination effect are all under investigation.

To summarize, it is an effective method of studying heavy metal pollution in order to further research the mechanism of heavy metal detoxification tolerance, isolate heavy metal resistance genes, and analyze the key causes of plant heavy metal resistance [97]. The detoxification mechanisms of heavy metal transporters, antioxidant enzyme systems, cell walls, and vacuoles in plants must be studied in order to provide a reference for future detoxification and tolerance mechanisms in plants.

1.3. Effects of Cd on germination, growth and development of winter wheat

Wheat, rice, and maize are the world's most important food crops. Wheat is a staple food for more than half of the world's population, with an annual global output of about 650 million tons. In other words, wheat is the main source of Cd intake for human.

Compared with other cereals, wheat mainly accumulates Cd through the root system, migrates to the above - ground portion, and finally accumulates in the wheat grain [98 - 101].

According to Lopez - Luna J. [102], compared to other hazardous metals, Cd is more harmful to wheat. Wheat absorbs and transports critical elements less effectively due to cd toxicity. Wheat's root shape and growth are severely impacted, which reduces plant growth, biomass, and grain output [103 - 111]. The issue of minimizing Cd contamination must be resolved immediately.

In recent years, agronomic management techniques have been tried to limit Cd uptake and toxicity in wheat, including the use of plant growth regulators (PGRs), mineral nutrients, biochar, fertilizers, compost, crop rotation, cropping patterns, and microbes [112 - 115]. However, these measures could pose some problems, such as large investment, high energy consumption, difficult operation and ease of secondary pollutant production [116]. Therefore, to ensure food safety, it is very important to study the molecular mechanism underlying the absorption, transport and efflux of Cd from wheat and to develop wheat varieties with low Cd accumulation.

The detoxification mechanism of heavy metals by plants, separation and accumulation of Cd absorption or cloning of functional genes, reveal the accumulation of low accumulation or not of grain crops, the molecular mechanism of Cd absorption, transport and accumulation, can clear the key process plant absorb Cd, its accumulation in the crop resistance control, reduce heavy metal consumption risks.

The seed germination and seedling stages are the beginning of plant life cycle. The seed is the first organ to comes into contact with Cd in soil. As a result, seed germination is the earliest stage to detect Cd toxicity.

In general, low Cd concentration has little inhibitory effect on seed germination and even promotes germination in some wheat varieties. With the increase of treatment concentration, Cd has a very strong negative effect on seed germination, inhibiting the growth of shoot and root system of wheat seedlings and decreasing dry matter accumulation [117].

The research demonstrated that wheat growth and the accumulation of dry matter were promoted by the treatment concentration of 0.03 mg / kg Cd. Wheat's development and accumulation of dry matter were drastically inhibited when the Cd content exceeded 0.03 mg / kg [117].

Amylase activity and starch hydrolysis in wheat cotyledons are inhibited by the buildup of Cd close to the radicle's growth point. As a result, the radicle and hypocotyl do not receive the nutrients they need to grow, which inhibits their elongation.

According to Sfaxi - Bousbih et al. [118], the soybean cotyledon to cotyledon

and radicle transport of minerals and carbohydrates was hindered, which had an impact on the germination and growth of seeds. The toxicity of cadmium rises as concentrations rise. Cd can stimulate plant growth at specific concentration levels.

However, as the concentration of Cd rises, Cd severely inhibits growth and development, which typically manifests as plant short stature, dechlorination of leaves, sluggish growth, and a loss in biomass. Plant photosynthesis, membrane function, in vivo enzyme system, and metabolism associated to physiological activities are all impacted by cd, which finally results in a loss in growth and yield [119 - 122].

In wheat leaves and other vegetative organs under Cd toxicity, the amount and rate of pre - flowering storage compounds were dramatically reduced, and the thousand seed weight also fell with an increase in treatment concentration. During the period from the young spike phase to heading, Cd poisoning prevented the differentiation of reproductive organs, which led to caryatization and abortion. Following heading, Cd poisoning prevents the production of chlorophyll, soluble sugar, soluble protein, and starch in wheat flag leaves and hinders the movement and redistribution of nutrients throughout the wheat plant [24, 123].

1.4. Cd uptake, distribution and Cd tolerance mechanism in wheat plant

Cd is highly toxic metal that influences plant growth and development through a number of mechanisms such as water nutritional balance and the production of reactive oxygen species [124]. Soil salinity, among other edaphic factors, may shift the soil - solution chemical equilibrium in favor of more soluble Cd compounds like

$CdCl_2$, thereby increasing its availability to plants [125 - 126]. These compounds' lower soil adsorption ability than free Cd ions increases Cd mobility at the soil - root interface. Furthermore, these complexes can improve Cd transport across plasma membranes, resulting in increased soil - plant Cd transfer under salinity [127]. In wheat, combined NaCl and Cd stress increased plasma membrane permeability and increased the production of oxygen radicals and H2O2 compared to Cd and NaCl treatments alone [128, 129].

Cd is generally absorbed by plants through the root system. The absorption of Cd by wheat roots at low concentrations was studied, and the energy required for transport was provided by the hydrolysis of ATP produced in the metabolic process, which was mainly reflected in the highly selective ion absorption and the energy consumption mechanism [130].

Plant root systems contain a variety of carrier proteins. Each ion binds to its corresponding carrier protein (transporter) to form ions carrier complexes that transport ions into cells using metabolic energy. At high Cd concentrations, absorption is a passive process involving diffusion, ion exchange, and chelation. Cadmium - containing plants have cationic exchange between the internal tissues of the root and the rhizosphere. Without Cd solution, desorption from the epidermal cell walls can occur. The other part is combined into irreversible macromolecular ules, after which Cd is absorbed at the root surface. The greater the proportion of large molecules, the longer combined into irreversible.

The process of diffusion is the entry of Cd into the cell through the cell wall and cell membrane, which is energy independent and dependent on the difference in

medium concentrations [131 - 133].

The uptake of Cd by wheat is also affected by root exudates and a series of changes caused by root exudates. Root metabolite release influences Cd uptake in wheat by influencing pH and Cd availability [134 - 137].

Furthermore, it has been reported that Cd uptake in the overground part of wheat varied according to growth stage, with the late growth period being larger than the early growth period, the growth flourishing period being larger than the slow growth period, and the reproductive growth period being larger than the vegetative growth period [138]. The amount and rate of Cd absorption at the jointing and heading stages were significantly higher than in previous periods. Cd absorption was significantly positively correlated with increase in dry matter weight ($r = 0.91633$), and absorption rate was significantly positively correlated with increase in dry matter weight ($r = 0.8003$).

Cd accumulation in wheat is dependent on transport from root to stem, whereas Cd accumulation in seeds is dependent on the transport from root to stem and direct transport of Cd from root to stem and grain occurs through xylem and phloem [138]. Cd concentration in wheat grains is determined not by xylem concentration, but rather by the ability of Cd to be transported from xylem to spike phloem [133, 138].

Cd could be transported from the applied leaves to other phloem reservoir organs, such as new leaves [39]. It was found that metal was transported in the soybean xylem in the form of cationic complex. Xylem contains a large number of amino acids and organic acids, and the metal complex formed by their combination

with metal ions can avoid the obstacles to positively charged metal transport caused by xylem cells' strong cation exchange ability, making it easier to transport [135].

Citric acid, low molecular weight dicarboxylic anions, and inorganic cations in xylem fluid flow can all affect Cd transport. Citric acid can promote Cd transport in xylem while reducing Cd transport out of xylem [136, 137].

Cd can entry to the plant via the xylem via symplastic transport and apoplastic transport under high exposure [138]. The phloem of flag leaves was primarily responsible for accumulation of Cd in the grain. Cd in the leaves and stems can be redistributed to the seeds, but Cd from the seeds is rarely transported to other parts of the plant. It can be speculated that Cd transport is related to the transport of photosynthetic products.

Metal ion re - transport into grains is also related to other metal ions. Zinc, for example, inhibits phloem loading and transport of Cd, reducing Cd transfer from phloem transport to grains. J. J. Hart [99] discovered that Cd transport to wheat seeds was possibly related to phloem-mediated Cd transport to the grain.

The phloem is responsible for Cd transport into wheat seeds. Because of its high mobility, Cd can easily reach plants via root uptake and translocation to stems and seeds [138]. Cadmium, as previously stated, enters the plants through the root system and is transported to the shoots in ionic form through xylem and phloem over transporters and transpiration.

Cd can have a wide range of effects on plants, including oxidative stress and nutrient uptake imbalance [139]. It can have an effect on the plant's the antioxidant defense system and induce the formation of reactive oxygen species (ROS), which

causes oxidative stress in general. After being absorbed from the soil by the plant, Cd accumulates in the roots or is transported to the stems, leaves, fruits, and other organs [140].

However, Cd accumulation in plants varies between organs, varieties, and ecotypes of the same species. Cd accumulation caused by roots is usually greater In the same plant object than in the stems, leaves, and grains. Cd is primarily distributed in the plastids and cell wall, and some of it precipitates as carbonate and phosphate. Wheat plants' leaf, root, and stem are easily enriched with Cd, whereas lower Cd levels in seeds are an immovable element that accumulated more in senescent parts and could not be reused by other non-senescent organs.

The filling and jointing - heading periods are critical for controlling Cd accumulation. At the early stage of filling, Cd accumulation in wheat organs is mainly higher in leaves than in the stems, leaf sheaths and grains. The accumulated content of the mature leaf was higher than that of the leaf sheath, grain and stem [140].

Under the Cd stress, it can stimulate plants' antioxidant defense system, remove O^2 - and H_2O_2, maintain the balance of reactive oxygen metabolism, and protect the membrane structure, allowing plants to endure, reduce or resist stress injury to a certain extent. As a result, one of the main mechanisms of Cd tolerance in plants, including wheat, is increased antioxidant enzyme activity [24, 129]. Enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) are examples of antioxidant defense systems, as are non - enzymes such as ascorbic acid (AsA) and glutathione (GSH). The method of direct Cd stress on wheat seeds

during germination was used, and it was discovered that the activities of superoxide dismutase (SOD) and peroxidase (POD) increased with concentration, as did malondialdehyde (MDA) content and cell membrane permeability. The activity of POD and CAT increased as stress intensity increased, indicating that the protective enzyme system in wheat was altered, as was the plant's protective response to the adverse environment [141 - 143].

Ascorbate peroxidase (APX) and glutathione reductase gene (GR) activity decreased as a result of the Cd stress because the wheat plant's ability to scavenge reactive oxygen radicals was compromised.

In their study, Wu et al. [144] demonstrated that Cd stress caused a significant reduction in GSH content in barley seedlings. It may have large-scale Cd detoxification, such as GSH for plant chelating peptide (PC) synthetic substrates, or it may have acted as antioxidants by removing biologically active oxygen free radicals and producing oxidized glutathione (GSSG).

Malondialdehyde (MDA) is a major byproduct of membrane lipid peroxidation, and its concentration is an important indicator of the degree or strength of peroxidation. MDA content in functional leaves of barley increased with Cd treatment and increased with the length of the Cd treatment time. Furthermore, when the concentration of Cd was greater than 0.1 mol, Cd stress would cause lipid peroxidation in barley leaves, whereas lipid peroxidation was alleviated after a certain period of treatment under low concentration Cd stress [144].

1.5. Main approaches and methods of winter wheat breeding

Traditional breeding methods

The CIMMYT wheat breeding program used genealogical breeding successfully in 1940. From 1985 to 1994, the modified genealogy method was the primary method, followed by the selective hybrid method. Single plant pedigree selection was used in F_2 and F_6 generations, mixed selection was used in F_2 and one F6 generation, and single plant pedigree selection was performed twice in one breeding cycle. The hybrid selection method, on the other hand, only performs single plant pedigree selection once in a breeding cycle, that is, the hybrid selection method is used in F_2 - F_5 generations and the pedigree selection is performed in F_6 generations. To study disease resistance, the modified pedigree method and selective hybrid method are frequently used in conjunction with backcross breeding. A single backcross combined with selective mixing successfully polymerized multiple resistance genes to stripe and leaf rust in wheat [145, 146].

In wheat breeding, backcross breeding is an important method of gene transfer. Single backcross was first used to improve rust resistance [147], which not only retained most of the major additive genes in recurrent parents, but also effectively aggregated the superior microgenes of donor parents, increasing the yield potential of backcross progenies. Reddy and Comstock [148] investigated the number of favorable alleles fixed by a single backcross breeding method and the effect of heritability, finding that the higher the heritability, the better the effect of backcross fixation on donor parents' favorable genes. However, the outcome was not as

anticipated. The efficiency of simple backcross transfer of one or two major genes can be identified using molecular markers [149 - 151]. They believed that multiple backcrossing could not only fully restore the recurrent parent background, but also introduce donor genes. In most cases, breeding traits are controlled by both major and minor genes rather than a single gene. However, because there are no closely linked markers for breeders to use, these traits must rely on phenotypic selection.

The improvement effect of multiple backcrossing on multi-gene controlled traits is not ideal. To further solve the breeding problem, several backcrosses can not only introduce the good genes of donor parents into recurrent parents, but also improve recurrent parents' adaptability. The study found that when the donor parent had low adaptability and more than three genes for good donor traits, or when the number of genes for good donor traits was less than or equal to three, two backcrosses produced better results. When the parent has general adaptability but more than three good donor trait genes, one backcross is best. Although breeding techniques have demonstrated that the improved variety adaptability is not significantly different after two and three backcrosses, genes for superior donor qualities may be lost as the number of backcrosses rises [152]. In addition, backcrossing beyond two generations is not necessary if there is no molecular marker available to track the target gene in the donor parent.

The early 1970s saw the adoption of triple-cross (or top-cross) and double-cross tactics by CIMMYT breeders in an effort to increase population genetic diversity [152]. The three-cross approach can be employed when there is a three-parent distribution of the target gene. In three-cross combinations like (Plxp2)

XP3, the genetic proportions of P_1 and P_2 each made up 25%, while the genetic proportion of P3 made up 50%.

In actual breeding, it is common practice to use parents with significant target qualities or varieties with great comprehensive traits as the parents of the final cross in order to improve the proportion of the parents' positive traits in the genetic makeup of the hybrid offspring. *Fusarium* head blight was to be improved via three crossings, first with two resistant parents and then with a third parent with superior adaptation, according to CIMMYT.

Marker - assisted breeding

More focus is being placed on the use of molecular markers to improve selection during breeding. Utilizing marker-based quantitative trait loci, it was possible to identify soybean germplasm in the early years of 1999. (QTL).

Bai Yi Xiong analyzed the genetic information of 113 barley materials by using QTL method [153]. A certain foundation has also been established for the creation of molecular markers associated to Cd accumulation capacity. Major genes associated with Cd uptake and transport in *Arabidopsis thaliana* have been identified as ABC family [154, 155], HMA family [156 - 160], Nramp family [161], ZIP family [162].

Theoretical support for the breeding of low Cd accumulation samples can be obtained by understanding the genetic foundation and gene composition of Cd absorption in wheat varieties. The backcross breeding method is restricted to the Scopc20 gene, a dominant marker linked to elevated Cd concentration [159, 163, 164]. An EST-derived marker (XBF474090) created by Wiebe et al. [115] was

co-isolated from a gene variation of the Cd-absorbing trait in crops and successfully converted into the co-dominant CAPS marker USW47.

Electrophoresis analysis of the PCR amplification products digested with Hpyl88 I restriction enzyme revealed that the marker may be used to identify two alleles of the Cd absorption trait genes. A total of 96 wheat samples were successfully classified as either having low or high Cd absorption. Kim et al. [165] isolated a *TM20* gene from a wheat root cDNA library that produced specific Cd (Cd (II) tolerance. These genes may be related to Cd accumulation in wheat and could be developed further to match molecular markers. Using molecular marker technology to screen germplasm resources is a novel concept. The presence of numerous polymorphisms in genomic DNA is used to develop molecular markers. It is a new and reliable genetic marker that directly reflects biological differences at the DNA level. The environment and developmental stage have no effect on DNA molecular markers, and a large number of markers can greatly improve the effectiveness and reliability of cross breeding [164 - 166].

Mark - assisted selection (MAS) is based on linkage imbalance between markers and Quantitative trait loci (QTL). If there is a strong enough linkage imbalance, the breeder can use a marker closely linked to a QTL to indirectly select that locus. Compared with traditional phenotypic selection methods, MAS has the following advantages:

1. When phenotypic identification of a trait is difficult, it is easier to use marker selection to modify the trait, such as root system, stress resistance and bread baking quality;

2. Phenotypic identification takes a long time;

3. When the cost of phenotypic trait identification is high, molecular marker selection is relatively cheap;

4. molecular markers can be used for selection in early generations.

The main MAS methods include:

1. Marker-assisted backcrossing (MAB): When the recipient parent needs to improve one or more adverse genes, the donor parent's beneficial gene can be introduced into the recipient parent through repeated backcrossing, and the target gene can be selected and the genetic background recovered using molecular markers;

2. Mark - assisted pyramiding or gene pyramiding, when the target gene is distributed between two materials (parents) or multiple materials, those materials are crossed, and molecular markers are used to screen and identify whether the hybrid population contains the target gene;

3. Mark - assisted recurrent selection (MARS), which involves marker - assisted selection of random mating populations over several generations.

In addition to the three methods mentioned above, molecular markers can also be used to aid in the identification of breeding materials for purposes such as determining variety consistency and purity, assessing genetic diversity and studying heterosis. Backcross breeding efficiency can be greatly improved by using molecular marker selection during backcross. Marker - assisted backcross consists of three stages.

In the first stage, target genes are selected using markers, namely prospect

selection [167]. When phenotypic selection was time - consuming and laborious, molecular marker selection was more effective. Recessive genes can also be selected in early generations. In the second stage, recombinon selection is the recombinon that the target gene exists in backcross progenies and the target site is exchanged with the linked marker. The aim of recombinon selection is to reduce the length of the donor fragment that contains the target gene. Using conventional breeding methods, donor fragments remain long even after many backcrosses. The length of donor fragment can be effectively reduced by using side linkage markers closely linked to target genes. The third stage is to select the offspring from recurrent parents who have the greatest degree of genomic recovery. Background selection is performed using markers that are not linked to target genes. Traditional backcross methods require at least 6 generations of backcross to restore the genetic background to that of recurrent parents, but the target gene still carries a long donor fragment. Marker - assisted selection, could save 2 - 4 backcross generations while reducing the target gene fragment.

Transgenic breeding

Traditional breeding methods have helped breeders produce high - yielding crops for centuries, but barriers to interbreeding between species have limited the development of new varieties. Transgenic breeding is a method that uses modern recombinant technology to transfer and communicate genes across species, regardless of species classification. Although there have been many research papers on GM breeding and many patents issued in recent years, only a few genes have been tested and evaluated in the field, and even fewer varieties have been cultivated

using GM [168]. Transgenic technology needs to be combined with traditional breeding methods and MAS technology to give full play to its due value. People use recombinant DNA technology to guide exogenous genes into plant cells, where they integrate, express and pass on to create new plants. The new plants created by this method are called transgenic plants. Using recombinant DNA technology can be improved crops proteins, essential amino acids content in crop, the fatty acid composition, antiviral, plant, in sect resistance and resistance to adversity makes plants in yield and quality, resistance and so on all have significantly improved, but also greatly reduces the agricultural production cost, alleviates the worsening Agricultural ecological environment.

Molecular design breeding

molecular breeding methods under the guidance of genetics and molecular biology and other theories, modern biotechnology methods are applied to the traditional breeding process, so as to breed new excellent varieties. In general, molecular breeding methods include marker - assisted breeding, transgenic breeding and molecular design breeding. In addition, genome - wide selection breeding can also be regarded as an important part of molecular breeding.

Although the traditional breeding method has played a great role in promoting the genetic improvement of crops. It still has many defects such as blindness and long time. These defects greatly limit the actual efficiency of crop genetic improvement. However, with the rapid development of modern molecular biology, especially functional genomics and bioinformatics, molecular design breeding came into being.

Crop molecular design breeding was first defined by Dutch scientist Peleman and Vander Voort [169], and later by Wan et al. [170]. They proposed that design breeding generally includes the following three processes:

1. Alleles and gene interaction information obtained by studying target trait genes, including mapping population construction and QTL mapping analysis;

2. According to the requirements of different environmental conditions and breeding targets, target genotypes are designed. This process requires the use of identified QTL information such as chromosome location, additive effect, interaction effect, etc.

3. Select the breeding program to achieve the target genotype, optimize it by using simulation method, and propose the best breeding program [171].

With the innovation and development of crop genetic material, such as the emergence of advanced construction group and derivative groups of parents to create more, and a large number of important traits genetic study to meet the requirements of breeders the development and application of computer aided Simulation tool, is to promote the application of design breeding practice in crop breeding, molecular design breeding system and the establishment of the support platform [172 - 175]. The study of genes controlling target traits and their relationship is the first step of molecular design breeding, and the most commonly used method for this research is QTL mapping. Molecular breeding is a new way to select germplasm resources [176 - 178]. When compared to conventional chemical analysis methods, molecular breeding produces no secondary pollution and it is the most effective and important mode of reducing Cd accumulation in agricultural

products. Using molecular breeding technology and successfully integrating it with traditional breeding methods to select crop varieties with low Cd accumulation will have a potential impact on the development of low Cd wheat germplasm and important practical significance for ensuring safe agricultural production of Cd contaminated soil.

MicroRNA (miRNA) is a new type of expression regulator that negatively regulates the expression of target genes after transcription by mediating the degradation or translation of target mRNA [179 - 180]. MicroRNA is central to gene expression regulation. With the development and application of high-throughput sequencing technology in recent years, an increasing number of miRNA related to heavy metals in plants have been cloned and identified. Previous research has discovered that these Cd-related miRNAs can participate in the response to Cd stress via heavy metal transport, sulfur assimilation, antioxidant stress, and auxin signal transduction pathways, and thus play an important role in the heavy metal stress response process of plants [181 - 183].

For example, MiR159 and MiR67 regulate heavy metal ion transport via the ABC (ATP - binding cassette) type transporter and the Nramp family (Natural Resistance - associated Macrophage Protein) of important proteins [184, 185]. MicroRNA-395 participates in the response to heavy metal Cd stress by regulating sulfate - starved low affinity sulfate transporters and the APS1, APS3, and APS4 genes (ATP s ulphurylase, APS) [184].

MiR398 plays an important role in the stress response of Cd, Hg, Cu and other heavy metals by targeting two kinds of SOD, namely CSD1 and CSD2 in Cu and

Zn superoxide dismutase (Cu, Zn superoxide dis - mutase, CSD) [184].

Natural resistance associated with macrophage protein (OsNARMP5) is a strongly expressed Cd and Mn transporter in the root of rice. The mutant of OsNARMP5 can significantly reduce the absorption of Cd by the root system of rice, thus reducing the content of Cd in the grain to below 3% of the control. OsHMA3 of the PIB - ATPase subgroup is a heavy metal ion pump mainly expressed in the root of rice, which is located on the vacuole membrane and mediated the enrichment of Cd in the vacuole of rice root cells. The over - expressed plants can selectively reduce the accumulation of Cd in seeds [185].

Low affinity cationic transport protein (LCT1) is a new transport protein cloned from wheat, which is mainly expressed in the root and leaf of wheat [186]. After RNA interference with OsLCT1 in rice, there was no significant change in xylem mediated Cd translocation.

However, phloem - mediated Cd translocation decreased significantly, and the content of Cd in seeds was reduced to half of the control, indicating it could be involved in the process of Cd transport from xylem large vasc ular bundles to dispersed small vasc ular bundles of phloem in stem and nodes as well as the process of phloem - mediated Cd transport to grains.

The results showed that the study of the genes related to Cd stress played an important role in the development of new varieties of Cd tolerant crops, which laid a foundation for excavating and functional analysis of Cd stress related genes in wheat [187 - 189].

Research in this direction provides insight into the screening of functional

genes that respond to stress, which can be used to analyze and improve crop resistance to Cd stresses. Simultaneously, it opens up a new way of breeding high Cd tolerance wheat varieties with high speed, simplicity, and low cost, which is critical to improving wheat grain safety and promoting the sustainable development of agricultural production [190].

1.6. Breeding strategies and possible schemes for Cd - low accumulation winter wheat varieties

The organization of selection work is based primarily on the world's genetic resources or collections of cultivated plants for the creation of sources and donors of selectively important traits. Winter wheat is the most important crop in Ukraine and the world, supplying human food needs [191, 192, 193].

Nowdays in the State Register of Plant Varieties in Ukraine, there are more than 460 varieties of winter wheat. For effective breeding work, the initial material must be studied in detail to meet specific parameters and requirements. The leading breeding institutions are studying wheat collections, which makes it possible to identify sources and donors of necessary breeding traits and involve them in hybridization [190, 194, 195]. Thanks to the successful work on the study of collections, wheat samples with a high level of homeostaticity, wide adaptability, group resistance to diseases and with high yield were isolated.

One of the current areas of breeding work is to obtain a source material with low ability to accumulate heavy metals, in partic ular cadmium. The minimization of Cd in wheat grain is urgently needed in many regions of the world. In many

countries technical solutions for decreasing wheat grain cadmium are elaborated. One of the possible ways to solve this problem is to create breeding varieties, which are characterized by low ability to accum ulate this heavy metal. Advances in genetics and molecular biology have expanded the possibilities for many modern selection methods that ensure wheat stability to Cd [194, 195].

Modern breeding tools also offer great potential for plant breeding programs that can be used alongside traditional breeding to create low Cd varieties [31, 196].

The potential of conventional breeding is still an attractive approach to modifying the Cd uptake of wheat varieties.

Manipulations with heterosis also open new perspectives for decreasing wheat Cd accumulatiom and adapting to Cd stresses. [10, 197]. However, there are several limitations to low - Cd wheat breeding because it is time - consuming and the genetic improvement process is rather slow [140].

The ability of wheat species and cultivars to absorb, accumulate, and tolerate Cd varies greatly [189]. Differences in Cd accumulation may also depend on the adaptation of different genotypes to environmental and production conditions. Low - Cd wheat cultivars are the most effective mean to reduce risks that are related to food consumption [198].

Creating of wheat Cd - tolerant varieties and reduction Cd accumulation in grain can be realized by both conventional and modern breeding methods. In conventional breeding, low - Cd wheat varieties are selected based on different traits (morphological, physiological, or biochemical) that are associated with Cd uptake. To improve the genetic background of wheat varieties with Cd tolerance,

intra - specific crosses among superior individuals are usually developed, followed by selection in subsequent generations. Breeding methods, such as mass selection, pure line, and recurrent selection methods can be used effectively to develop low - Cd wheat varieties [140].

Conventional selections are dependent upon environmental variations and thus require a widespread location field trial, delaying the progress of variety development [199].

As a general breeding criterion, it takes 8 - 10 years of significant breeding efforts to breed a cultivar from the pre-breeding phase to commercial release. Conventional breeding has been used successfully, and significant breeding progress has been made in many traits such as yield, quality, and stress resistance. When compared to breeding for other plant characteristics, using traditional methods for adaptation to abiotic stresses is difficult. Depending on the plant stress - adaptive nature, different resistance mechanisms exist for each of the abiotic stresses [200, 201].

Despite these difficulties, plant breeders generally used conventional breeding methods to solve this problem (development of low - Cd wheat cultivars), namely introduction, selection, and hybridization [140].

As a result, several low - Cd wheat cultivars were developed using traditional breeding methods. Yue et al. [200], for example, studied three wheat cultivars at four different Cd levels. JD 8 was identified as a Cd-tolerant variety, with the lowest Cd content and relatively less toxicity when compared to other cultivars.

Naeem et al. [201] tested 15 wheat cultivars at concentrations of 15, 30, and

45 μM Cd. The results revealed that Lasani - 2008 and Iqbal - 2000 exhibited the lowest Cd contents. Moreover, a large number of conventional studies were performed to screen out Cd - safe wheat cultivars.

Conclusions to Chapter 1.

There are practically no Cd - low wheat varieties in production but varieties with this characteristic are extremely necessary. It is desirable that such varieties combine resistance to Cd accumulation with such valuable trait as high yield capacity.

Conversional breeding is a time-consuming and lengthy process. However, these methods provide the initial material with the desired characteristics and the development of new varieties of winter wheat with low Cd - uptake. The achievements of traditional breeding in creating varieties with low Cd accumulation are convincing.

Biotechnology methods are relevant and promising as well. These methods significantly speed up the selection process.

A promising approach in modern breeding is the combination of molecular genetic developments with traditional breeding methods.

CHAPTER 2

CONDITIONS, MATERIALS AND METHODS OF RESEARCH

The research was conducted in accordance with the thematic plans of research works of Sumy National Agrarian University in the framework of the topic «Creating the source material of cereals and oilseeds resistant to the accumulation of heavy metals» (state registration number 0119U101581, 2019 - 2023).

The research, which was the base of dissertation consisted, of two experiments.

1. Study of the collection and obtaining the source material of winter wheat with low ability to accumulate cadmium. The experiment was performed on the basis of educational research and production complex of Sumy National Agrarian University (2019 - 2021) located in the north - eastern part of Forest - Steppe of Ukraine

2. The discovery and functional analysis of the *TaSFT2L* gene (2019 - 2022). This experiment was performed in China (Henan Institute of Science and Technology (Xinxiang, China).

2.1. Experiment 1. Study of the collection and obtaining of initial material of winter wheat with low ability to Cd accumulate

Study of the collection was carried out during 2019 - 2021, hybridization was done in 2019, collection of intervarietal hybrids was investigated for 2019 - 2021 in field condition.

2.1.1. Characteristics of the initial material

The collection of winter wheat (*Triticum aestivum* L.), which included 40 varieties, was formed on the basis of crop regional distribution and their yield in the demonstration field (Table 2 .1).

Table 2.1. Samples of winter wheat in a collection nursery

	Sample	Subspecies	Origin
1.	Alliance	ERSP	Plant production Institute named after V.YA. Yuriev
2.	Rozkishna	LUT	Plant production Institute named after V.YA. Yuriev
3.	Pryvitna	LUT	Plant production Institute named after V.YA. Yuriev
4.	Zdobna	LUT	Plant production Institute named after V.YA. Yuriev
5.	Pryvablyva	LUT	Plant production Institute named after V.YA. Yuriev
6.	Fortova	ERSP	Ivanivska experimental - breeding station
7.	Okhtyrchanka juvileina	ERSP	Ivanivska experimental - breeding station
8.	Zorepad bilotserkivskyi	LUT	Bila Tserkva experimental - breeding station
9.	Romantyka	ERSP	Bila Tserkva experimental - breeding station
10.	Shchedra nyva	ERSP	Bila Tserkva experimental - breeding station
11.	Tsarivna	ERSP	Bila Tserkva experimental - breeding station
12.	Lybid	LUT	Bila Tserkva experimental - breeding station
13.	Vidrada	ERSP	Bila Tserkva experimental - breeding station
14.	Oberig myronivskyi	ERSP	Myronivskyi Institute of Wheat named after Remeslo
15.	Svitanok myronivskyi	LUT	Myronivskyi Institute of Wheat named after Remeslo
16.	Kraevyd	ERSP	Institute of agriculture
17.	Rusyava	ERSP	Institute of agriculture
18.	Osyaina	SUB ERSP	Institute of agriculture
19.	Zaotar		Institute of agriculture
20.	Polisyanka	LUT	Institute of agriculture
21.	Rosynka	LUT	Institute of Irrigated Agriculture
22.	Konka	ERSP	Institute of Irrigated Agriculture
23.	Ovidiy	LUT	Institute of Irrigated Agriculture

24.	Mariia	ERSP	Institute of Irrigated Agriculture
25.	Kantata odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
26.	Sonata odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
27.	Duma odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
28.	Liga odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
29.	Oktava odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
30.	Optima odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
31.	Rodzinka odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
32.	Melody odes'ka	LUT	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
33.	Pylypivka odes'ka	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
34.	Hurt	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
35.	Rozkvit	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
36.	Sich	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
37.	Khvala	LUT	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
38.	Slaven	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
39.	Klad	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies
40.	Zorepad	ERSP	Selection - Genetic Institute - National Center for Seed Research and Variety Studies

The collection includes such varieties as: 16 – (originator –Selection - Genetic Institute –National Center for Seed Research and Variety Studies), 5 – (originator - Plant Production Institute named after V.YA. Yuriev), 6 – Bila Tserkva experimental - breeding station, 5 – Institute of Agriculture, 4 – Institute of Irrigated Agriculture, 2 – Myronivskyi Institute of Wheat named after Remeslo, 2 – Ivanivska experimental

breeding station.

In 2018 p. cross - breeding of different genotypes of winter wheat was carried out and received of 40 hybrid combinations. 14 samples and were used as initial material for cross - breeding. The selection of paternal parents was carried out according to previously obtained data from our study of the ability to accumulate cadmium and parameters of genotype productivity. The working collection was analyzed for the ability of plants to accumulate cadmium under the conditions of an analyzing background as well.

The brief description of parental form characteristics used in crossings are presented.

Sich

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Variety of intensive type, Steppe ecology, with a high level of adaptation to growing conditions. Medium - early type, growing period is 279 - 283 days. Plant height is 90 - 105 cm. It is resistant to lodging and shedding. Weight of 1000 grains is 42 - 43 g, yield is 8.67 - 10.25 t / ha.

Slaven

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Variety of intensive type and universal use. Early ripening type. Plant height is medium (90 - 105 cm). Weight of 1000 grains is 38 - 43 g, the average yield is 6,45 t / ha.

Okhtyrchanka juvileina

Originator is Institute of Sugar Beets of the Ukrainian Academy of Agrarian

Sciences, Ivanivska experimental breeding station. Erythrospermum subspecies. Variety of intensive type. It is characterized by unique combination of high productivity and resistance to extreme biotic and abiotic factors and excellent grain quality indicators. Medium - ripening type, growing period is 280 - 285 days. It is winter - hardy, resistant to lodging and shedding of grain, drought - resistant. Weight of 1000 grains is 39 - 45 g, Potential yield is up to 10,0 t / ha.

Zorepad

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Variety of intensive type, with genetically high yield potential. Early ripening type. Medium - sized plants with height up to 85 - 105 cm. Medium - early type. It is resistant to lodging, shedding, highly frost - resistant, drought - heat - resistant. Weight of 1000 grains is 38 - 42 g. Yield is 4,5 - 4,8 t / ha.

Duma odes`ka

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. The variety responds positively to a high agrobackground, at the same time provides high yields on a low agrobackground Medium ripening variety. Vegetation period is 286 - 287 days. Plants are with a short stem. It is resistant to lodging. The variety is drought - resistant, frost resistance and high winter hardiness. Weight of 1000 grains is 42 - 45 g. Yield is 6,6.2 t/ ha.

Rozkvit

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Variety with high disease resistance.

Frost resistance and winter hardiness is above average. It is drought resistance and heat resistance. The variety is resistant to lodging, shedding, and germination of grain in the ear. Height is medium (95 - 105 cm). Medium - early variety. Growing season is 278 - 284. Weight of 1000 grains is 39 - 42g. Yield is 8.03 - 10.86 t / ha.

Kantata odes'ka

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Intensive type of universal use on various agricultural backgrounds. It is of medium - sized (88 - 96 cm), highly resistant to lodging and shedding. Frost resistance and winter hardiness is high. Variety is characterized by high drought and heat resistance. High yields is provided by combination of high productive bushiness (680 - 850 stems per 1 m^2), large spikelets (10.1 - 11.8 cm length), with grains of 52 - 66 grains. Weight of 1000 grains is 38.4 - 46.3 g. Yield is 7.8 - 10.2 t / ha.

Кubok

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Middle - early type. Height is 90 - 115 cm. The variety responds well to basic fertilizers and top dressings by increasing grain yield and quality. It is drought and heat resistant. It is resistant to grain germination in the ear. It is resistant to lodging and shedding. Weight of 1000 seeds is 39 - 42 g. Yield is 9.2 - 10.2 t / ha.

Melody odes`ka

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Lutescens subspecies. Early - ripening type, growing season is

282 - 284 days. Frost resistance and winter hardiness is above average, drought resistance is extremely high. Yield is 7,2 - 11,4 t / ha.

Shchedra nyva

Originator is Bila Tserkva experimental - breeding station. Erythrospermum subspecies. Middle - early type. Growing season is 250 - 275 days. Plant height is 85 - 88 cm. Resistant to lodging and diseases. Weight of 1000 grains is 45 g. The potential yield of the variety is high. Yield is 6.6– 7.7 t / ha.

Svitanok myronivskyi

Originator is Myronivskyi Institute of Wheat named after Remeslo. Lutescens subspecies. Intensive type variety. Steppe ecotype. Irrigation is possible. The genetic potential of grain yield and quality is most fully realized on high agricultural backgrounds with intensive cultivation technology. Early ripening type. Plant height is 95 cm. It as winter hardy, drought - resistant and resistant to lodging. Weight of 1000 grains is up to 50,2 g. The maximum yield is 9,2 t / ha.

Oktava odes`ka

Originator is Selection - Genetic Institute – National Center for Seed Research and Variety Studies. Erythrospermum subspecies. Plant height is 104 - 112 cm. Early ripening type. It is winter hardy and drought resistant. Resistant to lodging. Weight of 1000 grains is up to 50,2 g. Yield is 7,8 - 11,9 t / ha.

Rusiava

Originator is Institute of Agriculture. Erythrospermum subspecies. High - growing awn variety that forms high - quality grains. The variety is medium - riping, growing season is 287 days. The shape of the bush is upright. Stem is thick, strong,

125 cm of hight. It is resistant to complex of diseases - septoria, brown rust, powdery mildew. Indicators of drought resistance and winter hardiness are high. Weight of 1000 grains is 47 g. Yield – 10,4 t / ha.

Ovidii

Originator is Institute of Agriculture. Lutescens subspecies. Variety is of intensive type for non - irrigated and irrigated agriculture, universal use on various agricultural backgrounds. Plant height is 100 - 105 cm. Medium - early ripening variety, vegetation period is 280 - 285 days. Resistant to lodging, scattering and germination of grain in the ear. Frost resistance is above average. Drought resistance and heat resistance are high. Weight of 1000 grains is 39 - 42 g. Yield potential – 9,5 - 10,0 t / ha.

2.1.2. Conditions of research

Soil and climatic conditions

The soils of the areas where the experiments were conducted are represented by typical heavy heavy - loamy medium - humus chernozem. The arable layer is of high quality, with a high humus content, an abundance of mobile forms of phosphorus and potassium, and a scarcity of nitrogen. There are following characteristics of it: humus content in the arable layer - 4.0%; The reaction of the soil solution is close to neutral (pH 6.5), the content of easily hydrolyzed nitrogen - 9.0 mg, mobile phosphorus and exchange potassium - 14 mg and 6.7 mg per 100 g of soils, respectively. This type of soil covers a significant part of the soil cover of the Forest - Steppe zone of Ukraine. The agrochemical characteristics of the arable soil layer are given in Table 2.2.

Table 2.2. Agrochemical characteristics of the soils of the experimental field

Indicator	Value
Score of soil quality, points	78 - 79
Humus content,%	4,1%
pH of the soil	6,5
Easily hydrolyzed nitrogen, mg/100 g of soil	11,2
Mobile phosphorus, mg/100 g of soil	11,3
Exchangeable potassium, mg/100 g of soil	9,2

The North - Eastern part of the Forest - Steppe of Ukraine is characterized by a temperate - continental climate and belongs to the zone of sufficient humidity. Climatic resources are important, for maximum realization of the crop biological potential.

The weather conditions of the research period were close to the long - term average with some tendency to warming and aridization.

The average long - term data on the dynamics of monthly temperatures and precipitation during the growing season are presented in Figure 2.1.

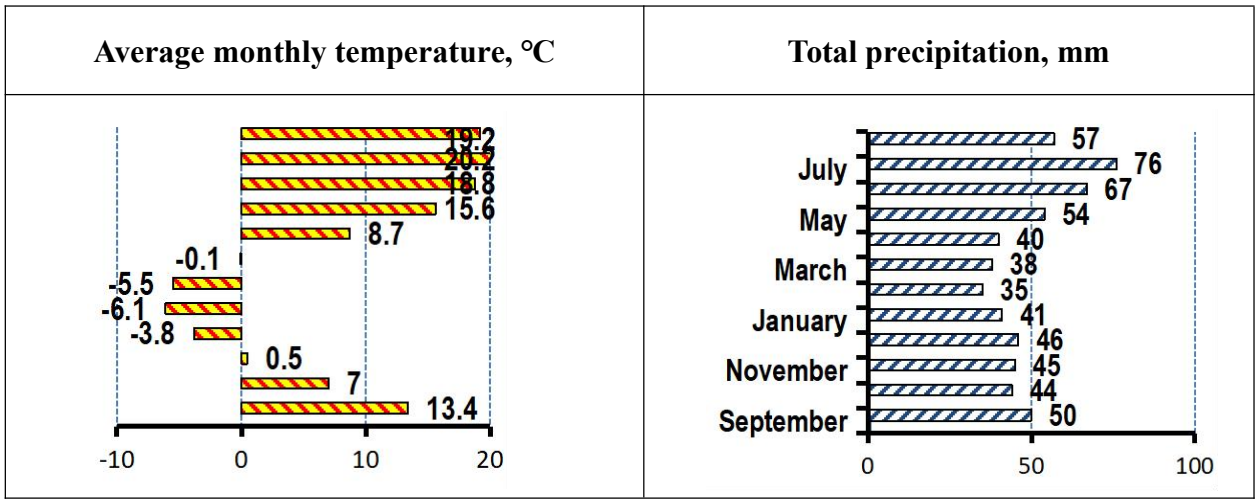


Figure 2.1. Long - term average monthly temperatures and precipitation in the study area (Meteorological Station of Institute of Agriculture of North - East of Ukraine).

The total indicators of the active temperatures sum (> + 5°C) during the agronomic year (from 01.09 to 31.08) of the region were 3754 °C.

The average annual air temperature was 7.4 ^{0}C. terminalation of autumn vegetation of winter crops was dated of 26 October. The optimal indicators for the beginning of winter wheat sowing were the second decade of September, the beginning of harvesting - the second decade of July. The total rainfall was 593 mm. The number of days with precipitation was 174. Steady snow cover was recorded from 01.12 to 10.04.

At average daily temperatures of September (13.4 and 7.0 °C in October, the sum of active temperatures of autumn vegetation is about 550 °C. These months were characterized by an average level of moisture supply. The amount of precipitation in September and October was 50 and 44 mm, respectively.

Average daily temperatures in the winter months range from - 3.8 in December

to - 6.1 in January. The last month is considered to be the coldest, with an absolute minimum of 36.0 °C.

In the spring the beginning of soil thawing is observed on 05.04. On the same dates there is a transition of average daily temperatures through the mark of + 5 °C and the beginning of the winter vegetation restoration.

The last month of spring (May) and summer months are characterized by the highest temperatures and maximum precipitation (more than 55 mm).This temperature dynamics provides the accumulation possibility of the sum of active temperatures at the level of 2200 - 2400 °C. by winter wheat during the autumn and spring - summer periods. However, deviations in the dynamics of temperatures (especially precipitation) cause fluctuations in yields.

Characteristics of the annual dynamics of temperatures and precipitation compa to long - term averages are given in (Appendix A, Appendix A1)

The graph data indicate the steady trend towards global warming and aridization in the area. Thus, the difference in the amount of precipitation compared to the average long - term values was minus 184, minus 127 and minus 141 mm in 2019, 2020 and 2021.

The average monthly temperature for the same period exceeded the normative values by + 2.2; + 2.8 and + 2.0 °C.

The average monthly temperature for the same period exceeded the normative values by + 2.2; + 2.8 and + 2.0 °C.

Differences in the quantitative indicators of heat distribution and precipitation in certain periods of development of winter wheat led to differences in the average

yield of the crop in the region, Figure 2.2. According to this yield, the most optimal weather conditions were in 2020.

The analysis shows that the features of the 2019 - 2020 agronomic year were satisfactory wintering conditions and low temperatures with sufficient moisture in the spring months.

These conditions ensured a high level of productive tillering and the realization of the varietal potential of the crop due to the high density of ears and higher values (compared to the average) of grain weight per ear.

Thus, field studies were carried out in typical soil and climatic conditions with slight temperature deviations

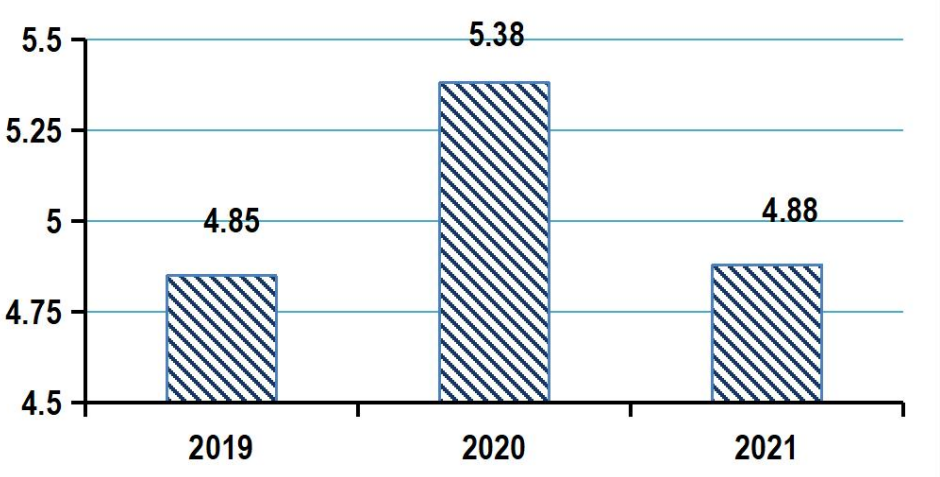


Figure 2.2. Average yield of winter wheat in the region of research. (https://superagronom.com/blog/778-agrorik-2020-v-tsifrah)

The growing season in 2020 was characterized by moderate temperatures and sufficient rainfall for growth periods. The sum of positive temperatures during the vegetation period was 2801.2 °C, the sum of active temperatures was 2907.0 °C, the amount of precipitation was 156.1 mm.

In general, the complex of hydrothermal conditions, the years of research were favorable for the growth, development and formation of a high level of productivity of winter wheat.

2.1.3. Methods of research

Soil prepearing included crumbling plant residues, harrowing, application of mineral fertilizers (nitroammophos at the rate of 200 kg per hectare) and plowing to depth of 22 cm. Plowing was carried out in the second decade of August. The predecessor was peas. Sowing was carried out in the last decade of September with a manual seed driller, with a sowing rate of 64 - 67 seeds per liner meter.

The duration of the autumn vegetation for the research years was 45 - 50 days. Spring fertilizer application was carried out in the 2 - nd decade of April (ammonium nitrate at the rate of 100 kg /ha). The total rate of fertilizers in the field experiment was $N_{63}P_{30}K_{30}$.

The plots had 3 rows 2 m long (area 0.9 m^2). The number of repetitions is 3.

F_1 -F_2 hybrids were sown with low plant density (distance between plants – 10 cm). Row length depended on seed number.

Biometric studies of plant parameters and analysis of crop structure were carried out for a sample of 25 - 30 plants (in each of the repetitions). Samples were formed from plants in the central row.

Analysis of the yield structure (height of plants, traits of the main ear - length, cm; number of spikelets, grain number, ear weight, 1000 seed weight) was carried out according to the recommendation [202].

The selection of sheaf samples was carried out in the phase of full maturity.

Cd content in root and shoot (winter wheat) was determined at stages of shooting. Subsamples were dried and then Cd concentration in them were determined by a spectrometer (CAS - 120).

Hybridization of plants was carried out under the conditions of a field experiment using the twel method. (2019) [203]. Castration was carried out in the central spikelets of the ear 2 - 3 days before the flowering beginning. Pollination was carried out on the 3 - 4 - th day after castration. The comparison of indicators for intervarietal hybrids (average for F_1 and F_2) was carried out to the standard. Conditional standard - average values for 3 varieties with a minimum level of cadmium accumulation: Shchedra nyva, Svitanok myronivskyi, Okhtyrchanka juvileina.

Inheritance coefficients and indicators of the degree of phenotypic dominance were determined according to the method of B. Griffing. [205]

The assessment of the range of dominance was carried out according to the scale G. M. Beil, R. E. Atkins, [206] where the numerical values were corresponded to the scheme:

Depression	Partial recessive dominance	Intermediate inheritance	Partial positive dominance	Dominance
< 1	- 0,5	0	0,5	1 >

Analysis of the degree of phenotypic dominance in selections of breeding

components was carried out in order to quantify the manifestation of traits for rapid assessment of hybrid offspring and improvement of traits.

Statistical processing of experimental data for generalization and definition reliability of the obtained results of studying the variability of morpho - physiological and parameters of productivity was carried out by using variation, dispersion and correlation analysis according to standard methods with using MS Exel 2010 software and Statistica [207, 208].

2.2. Experiment 2. The discovery and functional analysis of the *TaSFT2L* gene

2.2.1. Plant material, growth and Cd stress treatment

Plant material

The Bainong207 wheat cultivar was used for gene transformation and functional analyses of the *TaSFT2L* gene.

Bainong 207 is a wheat variety with high yield, stable yield, large ear, and strong disease resistance that was crossbred from Zhou 16 as the female parent and Bannong 64 as the male parent under the supervision of Henan Institute of Science and Technology's Professor Xingqi Ou. The total growth period is approximately 231 days, the yield is 8,25 - 11,25 t / ha, and cadmium resistance is low.

The WT was *Arabidopsis thaliana* ecotype Columbia (Col-0). One T-DNA insertion mutant of *AtSFT2* (vesicle transport protein of *Arabidopsis*, AT5G56020) was obtained from the AraShare (a non - profit *Arabidopsis* share center, http://www.arashare.cn), and designated as *Atgn9* (SALK_132905).

Growth and Cd stress treatment

Surface sterilization of the seeds for 6 min was done using 2.5% NaClO followed by 75% ethanol for 1 min. Then, seeds were washed using distilled water and incubated at 28 ℃ away from light for germination. After three days, partial seedlings were transplanted to a plastic plate with a sponge strip, after which they were transplanted to plastic containers (41 cm × 24 cm × 14 cm) containing 8 L Hoagland nutrient solution [210]. Treatment culture media was replenished every day. The other part of the seed was transplanted into black pots (10 cm × 24 cm × 14 cm), containing 500 g of nutrient soil.

Wheat seedlings were cultivated in a plastic container. For analysis of seedling responses to Cd stress, transgenic and wild - type (WT) seedlings (10 - day old) were subjected to 0, 0.5 mm, 2 mm and 10 mm Cd treatment [211]. After seven days, growth parameters, such as shoot and root length and chlorophyll contents were analyzed.

The mutant of *Arabidopsis thaliana* was grown in Murashige - Skoog (MS) medium containing 0, 50 uM and 75 uM Cd [212 - 213]. Root growth and fresh weight assays were performed.

2.2.2. Main reagents and enzymes

The work used reagents from the following companies.

1. RNA Extraction Kit (TaKaRa Company, Commodity No. 9769).

2. Reverse Transcription Kit (TaKaRa Company, Commodity No. RR047A).

3. GXL Hifi Enzyme (TaKaRa Company, commodity No. R050A).

4. Taq Enzyme (Beijing Kang Wei Century Biotechnology Co., LTD.).

5. Agarise Gel Recovery Kit (Tiagen Biochemical Technology Co., LTD.).

6. Plasmid Extraction Kit (Tiagen Biochemical Technology Co., LTD.).

7. Ampicillin (Solebao, commodity No. A8180).

8. *Smal*1 (New England Biolabs, Catalog number: R0141S).

9. *mlu*I (New England Biolabs, Catalog number: R0198S).

10. *Spe*1 (New England Biolabs, Catalog number: R0133S).

11. *Sac*1 (New England Biolabs, Directory Number: R3156S).

12. *BamH*1 (New England Biolabs, Directory number: R0136S).

13. *Kpn* 1 (New England Biolabs, Catalog number: R3142S).

14. *Xba*1 (New England Biolabs, Catalog number: R0145S).

15. Ehanol (Sigma - Aldrich, Catalog number: 459844).

16. Sodium acetate (Sigma - Aldrich, Catalog number: S2889).

17. Message mmachine T7 In Vitro Transcription Kit (Ambion Sigma - Aldrich, Catalog number: S2889).

18. Glycine (Sigma - Aldrich, Catalog Number: 410225).

21. Dihydroethidium (DHE) (Fluka Biochemika, Buchs, Switzerland).

22. DCF - DA (2 - 7′ - dichlorofluorescein diacetate, Calbiochem, San Diego, CA, USA).

23. DNA Gel recovery kit and plasmid small amount extraction kit were purchased from TIANGEN BIOTECH (BEIJING) CO.,LTD.

24. TaKaRa mini BEST Plant RNA Extraction Kit.

25. PrimeScript™ II 1ST Strand cDNA Synthesis Kit.

26. Rt - qpcr Kit (TB Green® Premix Ex Taq™ II (Tli RNaseH Plus).

27. T4 ligase, In - Fusion® HD Cloning Kit Seamless clonal enzyme; 28. Restriction endonuclease, PrimeSTAR® and GXL DNA Polymerase high - fidelity enzyme.

29. 2 * Tap Mastcr Mix, yeast transformation kit, were purchased from TaKaRa biotechnology co., LTD..

30. LB (Luria - Bertani) liquid medium formula: 5 g / L yeast extract, 10 g / L tryptone, 10 g / L sodium chloride, autoclave sterilization at 121 °C for 20 minutes. On the basis of liquid medium, solid LB medium was supplemented with 15 g / L agar powder. Antibiotics should be added to liquid LB medium after it has been cooled. Antibiotics should be added to solid media after sterilization and left for a period of time. Ampicillin, kanamycin, and rifampicin were used in the experiment, and the final concentration was 50 mg/mL.

31. SD liquid medium formula: 2% glucose, 1% peptone, 0.5% yeast extract, autoclave sterilization at 121 ° C for 15 minutes. On the basis of liquid medium, solid SD medium was supplemented with 15 g / L agar powder.

2.2.3. Strains and vectors

1. *Escherichia coli* 5a (DH5a) is a strain commonly used for plasmid cloning (SHBBY, Catalog Number: AS1.1145).
2. *Agrobacterium tumefaciens* (GV3101) is a strain, natural plant genetic transformation system (Waryong GT707).
3. Cd - sensitive mutant *ycf1*, yeast expression vector of pYES2.1.

4. Subcellular localization vector of pCEGFP.
5. Plant silencing expression vector of pTCK303.
6. Plant overexpression vector of pCAMBIA1301 were provided by plasmid platform Miaolingbio (http://www.miaolingbio.com/.).PMD - 19T vector was got from TaKaRa Biotechnology Co., LTD.
7. The pET - 28a - c(+) vectors carry an N - terminalal His•Tag®/ thrombin/ T7•Tag® configuration plus an optional C - terminalal His•Tag sequence.

2.2.4. Experiment equipment

- confocal microscopy (Carl Zeiss LSM780, Germany);
- fluorescence microscopy (Carl Zeiss Axio Zoom v16, Germany);
- electrophoresis apparatus;
- camera Canon, EOS M50, Japan;
- transmission electron microscopy (TEM) (Hitachi HT7700);
- spectrophotometr (TU - 1810, Puxi, China);
- emission spectrometer (ICP - AES).

2.2.5. Methods of research

Extraction of wheat DNA

Total DNA was extracted from young leaves using the Bu's method according to the instructions on the DNA extraction kit [213].

Extraction and reverse transcription of RNA

Total RNA was extracted from young leaves according to the instructions of

RNA extraction kit. The RNA obtained was reversed into single - stranded cDNA according to the instructions of the reverse transcription kit [213].

Cloning of TaSFT2L gene

Design of primers

Total genomic DNA extraction from leaves of *Arabidopsis thaliana* mutant plants during the rosette period was done using a DNA mini Kit (TIANGEN, Beijing, China), as recommended by the manufacturer. The primers IntronFw2 and NOS - 60 (Appendix D) were used to clone silenced genes that were outside of targeted gene regions for RNA interference. By using the DNAMAN software, the *TaBMY1* gene was translated into amino acid sequences. Phylogenetic trees were generated using MEGA 7.0 software after ClustalW alignment (http:// www.megasoftware.net).

The target gene was amplified by PCR.

The target gene was amplified with GXL hi - fi enzyme, and the PCR reaction system was as follows:

5×PrimeSTAR GXL buffer	10 μl
2.5 mmol/L dNTPs	4 μl
10 mmol/L forward primer	2 μl
10 mmol/L reverse primer	2 μl
cDNA as template	5 μl
PrimeSTAR GXL DNA polymerase	1 μl
RNase Free dH_2O	50 μl

The prepared reaction solution was mixed and then centrifuged for PCR amplification. PCR amplification conditions were as follows:

98 ℃ degeneration 10 sec ┐
60 ℃ anneal 15 sec ├ 32 cycle
68 ℃ extension 2 min ┘
4 ℃ preserved.

The amplified products were detected by 1.5% agarose gel electrophoresis. The DNA fragments were recovered by gel recovery kit.

Connection and transformation.

The target fragment was linked to pMD - 19T vector and thus transformed into *Escherichia coli* DH5a competent cells.

1. Connection system was:

pMD - 19T vector	1 μl
pMD - 19T - SFT2L fragment	4 μl
Solution 1	5 μl
Total volume	10 μl

2. After absorption, beating and mixing, the connection system was placed in a 16 ℃ metal bath for 30 min, and the competent of *Escherichia coli* was transformed by heat shock method.

3. The above connecting solution was added to 50 ul *Escherichia coli* DH5a induced state, then mixed gently, and placed on ice for 30 min.

4. Heat shock of 90 sec in the metal bath at 42 ℃ was done, then it transfered to ice for 2 min, then 800 ul blank LB medium into the centrifuge tube was added.

5. The centrifugal tube was shaken at 37 °C for 45 minutes. Following that, 100 ul of bacteria solution was evenly coated on an ampicillin-containing LB plate, sealed with sealing film, and placed in a 37 °C incubator for overnight culture.

Colony PCR identification.

Single colonies were selected from the ultra - clean workbench (marked) as templates, and colony PCR was performed to preliminarily identify positive clones.

The reaction system was as follows:

10 mmol/L forward primer	2 μl
10 mmol/L reverse primer	2 μl
cDNA template	4 μl
2*Ex Taq enzyme	25 μl
RNase Free dH_2O	50 μl

The PCR program was set as follows:

95 ℃ predegeneration 5 min

95 ℃ degeneration 20 sec

60 ℃ anneal 15 sec } 22 cycle

72 ℃ extension 1 min

72 ℃ eventually extend 2 min

4 ℃ preserved.

The amplified fragments were subjected to agarose gel electrophoresis.

Construction of the vector

Construction of PYES2 vector

1. The PYES2 vector plasmid was digested with *BamH* I and *EcoR I* (Figure 2.3), and the digested solution and the amplified target fragment were separated using agarose gel electrophoresis.

2. The ligating solution was connected using a seamless connection kit and then transformed into an *E. coli* receptive state before being coated on an LB plate containing Kanamicin (Kan).

3. The bacterial plaque was chosen for colony PCR verification, and the correct colony plasmid was confirmed by shaking and double digestion.

4. The correct plasmid would be sequenced for further confirmation. The recombinant plasmid was labeled *pYES2 - TaSFT2L*.

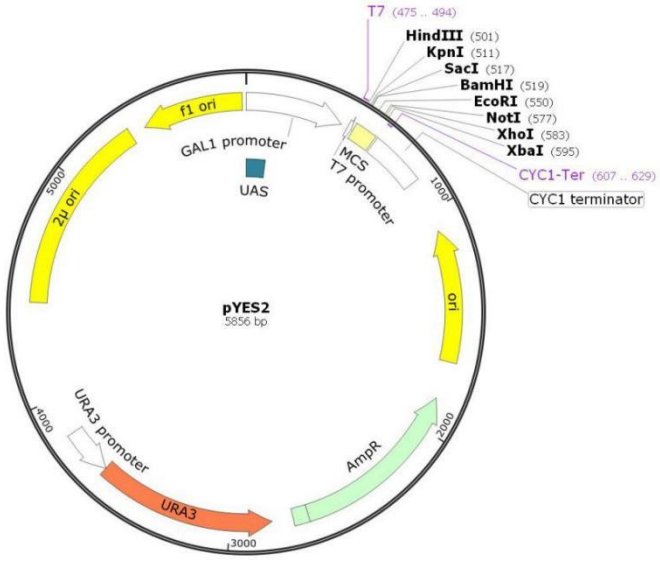


Figure 2.3. Plasmid map of pYES2.0 yeast expression vector.

Construction of Barley stripe mosaic virus (BSMV) - VIGS vector

The cDNA fragments of the phytoene desaturase gene (*TaPDS*) and *TaSFT2L* were amplified by oligonucleotide primers with *Sma*1 sites that had been reverse - inserted into RNAγ of BSMV to establish BSMV cDNA clones: The *TaPDS* and BSMV: *TaSFT2L* for gene silencing. In vitro transcription assays and intermixture formulae of RNAβ, RNAα, RNAγ and RNAγ - derivative clones were performed as suggested by Gunupuru et al. [224].

Construction of TaSFT2L gene expression vector in wheat

(1) Construction of RNA interference (RNAi) vector

- DNAMAN 6.0 software was used to analyze the enzyme site sequence of TaSFT2L gene, and primers (Appendix D) were designed based on the polyclonal site of RNA interference vector pTCK303.

- The *TaSFT2L* gene was amplified by Appendix D primers with *Spe*1 and *Sac*1 restriction endpoints (Figure 2.4). The same restriction endonuclease was used to digest the pTCK303 vector. *TaSFT2L* gene was amplified and purified by gel electrophoresis. The recovered product of plant RNA interference (RNAi) vector pTCK303-TaSFT2L and the recovered product of *TaSFT2L* silencing gene bound to Spe1 and Sac1 terminal sites were transformed into *E. coli* DH5α receptor cells, which were coated on LB solid medium containing kanamycin. Cultured at 37 ℃ for 12 - 16 h, positive clones were screened out.

- The *TaSFT2L* gene with the reverse fragment of the *BamH* I and *Kpn* I sites was amplified, and the pTCK303-TaSFT2L-RNAi plasmid was digested with BamH I and Kpn I (Figure 2.4).

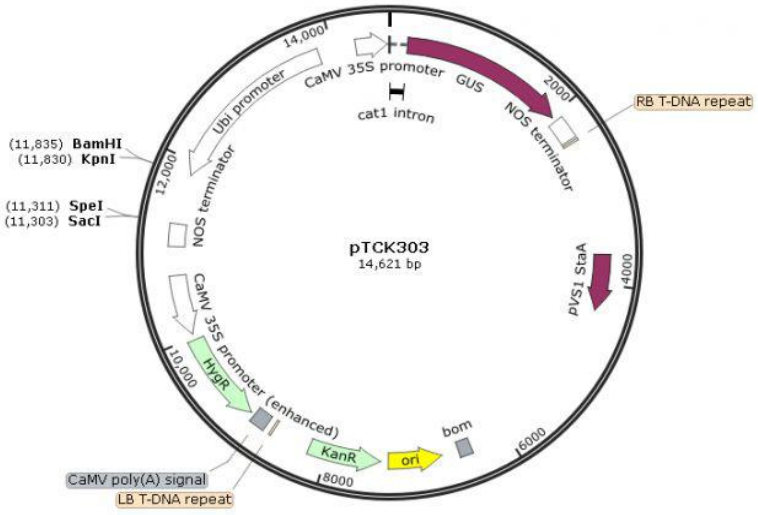


Figure 2.4. Plasmid map of pTCK303 expression vector.

(2) Construction of overexpression (OE) vector

- After analyzing the restriction sites of *TaSFT2L* gene sequence with DNAMNAN 6.0 software, primers (Appendix D) with *Xba* I and *Sac* I restriction sites were designed by combining the polyclonal sites of plant overexpression (OE) vector pCAMBIA1301 (Figure 2.5).

- *TaSFT2L* amplified by primers was linked to the recovered product of pCAMBIA1301 vector digested by the same enzyme. Enzyme digestion was carried out in the same way.

- The ligands were transformed into *E. coli* DH5α cells and coated with LB solid medium containing kanamycin. Cultured at 37 ℃ for 12 - 16 h, positive clones were screened for monoclonal identification.

- After monoclonal identification, plasmid was extracted by recombinant vector. The recombinant plasmid was identified by *Xba* I and *Sac* I double

digestion. The empty vector pCAMBIA1301 was used as control. The vector was named as *pCAMBIA1301 - TaSFT2L*.

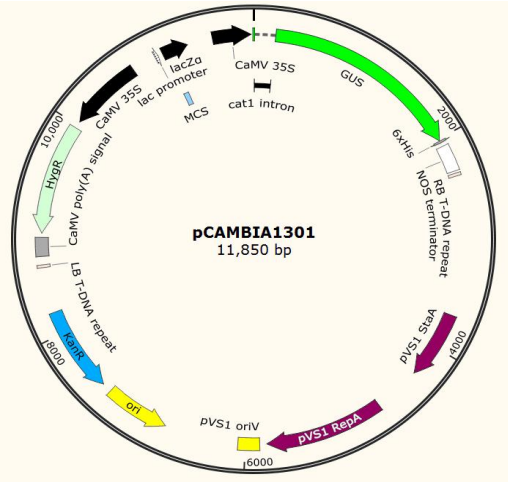


Figure 2.5. Plasmid map of pCAMBIA1301 expression vector.

(3) Construction of prokaryotic expression vector

1. The prokaryotic expression vector pET - 28b (Figure 2.6) was digested by *Bam*H 1 and *Hind* III vectors, respectively.

2. The purified *TaSFT2L* target fragment was connected to the pET - 28b vector, and the construction of *pET - 28b - TaSFT2L* vector was completed.

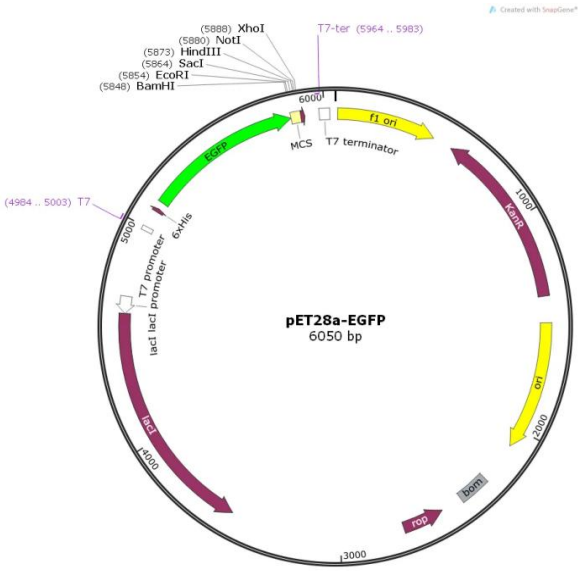


Figure 2.6. Plasmid map of pET - 28b expression vector.

Gene transformation

Preparation and transformation of receptive state of yeast.

- The original strain of *ycf1* was streaked in YPDA solid medium. After dark culture at 30 ℃ for 3 days, a single colony was selected and inoculated into 2 ml YPDA liquid medium for overnight shaking culture at 110 rpm.

- The 0.5 ml overnight culture solution was transferred to a 50 ml YPDA liquid medium and cultured at 120 rpm at 30 ℃ to achieve an ODA600 of 0.15 - 0.3. The bacteria solution was divided into a 50 ml centrifuge tube. The supernatant was discarded, and the bacteria were re- suspended in 20 ml of sterile ultra - purified water and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded after centrifugation.

- The cell suspensions were re - suspended by 1.5 ml 1.1 X TE / LiAc. The cell suspensions were separated into two 1.5 ml centrifugation tubes for 15 s at high speed. After supernatant discarding, the bacterial precipitate was re - suspend with 600 μ1 1.1 x TELiAc, and stored in 100μ1 per tube.

- Then cell suspensions was added of 240 μ1 50% PEG, 36μ1 1.0 mol/L lithium acetate, 25 μ1 salmon sperm DNA, 5μ1 plasmid, and swirled for 1 min to mix. The mixture was kept in a water bath at 42 ° C for 45 minutes, stirring up and down repeatedly every 7-8 minutes. After centrifugation at 3000 rpm for 5 - 10 min, the supernatant was absorbed with a pipette, and 200 μ1 $NaCl_2$ solution (0.9%) was added to resuspend the thalli. Then all the thalli were coated in SD - U solid medium for culture. After dark incubation at 30 ℃ for 3 days, single

colonies were selected and identified. After successful primers identification, the strains were preserved.

Plasmid DNA was transformed into Agrobacterium strains

The successfully identified of *pTCK303 - TaSFT2L* and *pCAMBIA1301 - TaSFT2L* plasmids as well as pTCK303 and pCAMBIA1301 empty plasmids were transformed into *Agrobacterium tumefaciens* receptor cell of GV3101. After transformation, the plasmids were coated on plate containing kanamycin and rifampicin resistance. The monoclonal clones were identified. After successful identification, the positive strains were identified and preserved.

Plant transformation

To obtain transgenic wheat plants after cloning of the coding sequence (CDS) into the pCAMBIA1301 and pTCK303 vector, it was connected to the *CaMV 35S* promoter. Recombinant vectors were relocated to GV3101 strains. *Agrobacterium* - associated transformation was conducted. The transgenic plants were provided by Wuhan Boyuan Biotechnology Co., LTD.

qRT - PCR analysis

Total RNA from roots, leaves, and other tissues were extracted using the Trizol reagent (TIANGEN, Beijing, China). Then, cDNA synthesis was done using the PrimeScriptM RT Reagent Kit with gDNA Eraser (TAKARA, Japan). Transcript levels of *TaSFT2L* in wheat were evaluated by qRT - PCR with TB Green™ Premix Ex Taq™ II (TaKaRa, Japan), using the ABI 7500 Real - Time PCR System.

Actin genes were used as controls in this assay. Appendix D (Appendix) shows the primers for this assay. The qRT - PCR procedure was conducted as described by Liu et al. [211, 223].

2.2.6. Experimental design

Screening of Arabidopsis RNAi library

The RMHR - based vesicle transport protein - inducible hairpin RNA library was constructed as reported by Wang et al. [202]. Using the floral dip method, transformation of the hairpin RNA library into *Arabidopsis* (Col - 0 ecotype) was done using of *Agrobacterium tumefaciens* GV3101 [213]. To obtain plants with the ability to alleviate Cd - associated toxicity, primary transgenic plants were screened using the MS medium supplemented with 0.1 mm $CdCl_2$ and 50 mg / L hygromycin.

Bioinformatics analysis of TaSFT2L gene.

Conserved domains of *TaSFT2L* were determined using the NCBI conserved domain search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

MEGA5 (http:// www.megasoftware.net) was used to generate phylogenetic trees [214].

Multiple alignments were generated using CLUSTALW as reported by Thompson et al. [214], with slight manual adjustments for alignment optimization.

SignalP 4.1 software /SignalP - 4.1/) was used to predict protein signal peptide.

Expression patterns of TaSFT2L gene in wheat under cadmium stress at different times

Seedlings with the same growth potential were selected and treated with 1.0 mm $CdCl_2$. After cadmium stress of 0 h, 3 h, 6 h, 12 h and 24 h, leaves were taken and treated with liquid nitrogen before being stored in a refrigerator at - 80 ℃. According to the coding sequence of the cloned *TaSFT2L* gene, primers for specific PCR were designed using Primer Premier 6.0 in the conserved region (Appendix D). The expression level of *TaSFT2L* gene was analyzed by qRT - PCR. The $2^{-\Delta\Delta CT}$ method was used to analyze the gene expression characteristics of *TaSFT2L* gene at different time under cadmium stress in wheat.

Samples of young roots, young leaves, stems, leaf sheaths, stem nodes, stamens and pistils of normal wheat plants were taken, and immediately frozen in liquid nitrogen (- 80 ℃). RNA was extracted from the above tissue parts of wheat and reversely transcribed into cDNA. Gene expression characteristics of *TaSFT2L* gene in different tissue parts of wheat were analyzed by qRT - PCR.

Subcellular TaSFT2L localization

The *35S - TaSFT2L - GFP* fusion vector and *35S - TaPDA62 - 1301 - RFP* (cytomembrane marker) were transferred into wheat epidermal cells via the protoplasts of wheat mesophyll cells [211]. After 72 h, fluorescence signal imaging was done by confocal microscopy (Carl Zeiss LSM780, Germany).

Determination of growth curve of yeast converters treated with Cadmium

Yeast cell densities were evaluated at various times (0, 2, 4, 6, 8, 10, 12 h) using a spectrometer at OD_{A600} = 0.1. To assess Cd accumulation in cells, *ycf1* yeast mutants had been transformed with TaSFT2L - pYES2 and pYES2 were grown in SD - Ura broth (50 ml) to OD_{A600} = 0.1.

They were subjected to 10 µm Cd treatment and they grown at 30 ℃ for 2 days. Using distilled water, yeast cells were washed four times, dried for 2 d at 75 ℃ after which Cd concentrations were determined by inductively coupled plasma mass spectrometry (ICP - MS), as reported by Zhang et al. [216, 217].

The autophagy complementation test

The autophagy complementation test was conducted as reported by Fujiki et al. [218]. The LiAc - induced assay was performed to introduce recombinant vectors (*TaSFT2L - pYES2* and pYES2) into *SFT2L* mutant yeast cells. Positive transformation was evaluated by SG - Ura, after which incubation under shaking was done at 30 ℃ in synthetic dropout medium (SD) medium to OD_{A600} = 1. Then, cells were obtained, washed, followed by incubation for 5 h in YNB medium (nitrogen - deficient) in the presence of 1 mm phenylmethyls ulfonyl fluoride (PMSF) for induction of autophagy and accumulation of autophagosomes in vacuoles. Cells were observed by differential interference microscopy (DIC, DM5000B, Leica, Germany).

Barley stripe mosaic virus (BSMV) - VIGS inoculation and TaSFT2L function analysis

The cDNA fragments of the phytoene desaturase gene (*TaPDS*) and *TaSFT2L* were amplified by oligonucleotide primers with *Sma* 1 sites that had been reverse - inserted into RNAγ of BSMV to establish BSMV cDNA clones. The TaPDS and BSMV: *TaSFT2L* is used to verify gene silencing. In *vitro* transcription assays and intermixture formulae of RNAβ, RNAα, RNAγ and RNAγ - derivative clones were performed as reported by Gunupuru et al. [224]. For inoculation, 10 ml of the intermixture were gently rubbed on the 2 - nd leaf surface of the two - leaf stage plants. After five days of post inoculation, qRT - PCR was conducted to verify transcript patterns of *TaPDS* and *TaSFT2L*. Then, for 14 days, they were treated with 0.5 mm of $CdCl_2$. Maximum root lengths were determined before and after treatment. Roots and leaves of the inoculated seedlings were separately harvested, rinsed using deionized water for physiological analyses and dried for 72 h at 80 ℃ to the constant weight. Treatments were repeated six times. The primers for this assay are shown in Appendix D.

Physiological analysis

Root activities

Root activities were evaluated by triphenyl tetrazolium chloride (TTC) method, as described by Zhang et al. [229].

Cell viabilities

To determine cell viabilities, 10 mm root segments were stained using FDA - PI (fluorescein diacetate (5 mg / ml $^{-1}$) and propidium iodide (2 mg / ml $^{-1}$) as reported by Jones and Senft [230]. Stained root sections were observed and imaged by fluorescence microscopy (Carl Zeiss Axio Zoom v16, Germany).

O•− and H_2O_2

Root segments (10 mm) were sliced from the apex, after which superoxide radicals (O•−) and hydrogen peroxide (H_2O_2) were respectively stained using of 10 μm dihydroethidium (DHE) (Fluka Biochemika, Buchs, Switzerland) and 25 μm DCF - DA (2 - 7′ - dichlorofluorescein diacetate, Calbiochem, San Diego, CA, USA), as reported by Sandalio et al. [220]. Then, after 30 min of incubation, DCF - DA and DHE - labelled root segments were evaluated in the corresponding stereomicroscopes (at 485 and 488 nm excitations and 530 and 520 nm emissions, respectively) via stereoscopic fluorescence microscopy (Carl Zeiss Axio Zoom v16, Germany).

Root surface morphologies

Root tips were prepared as described by Pan et al., [232] after which they were subjected to SYCOP3 (JSM - 6390/LV; JEOL, Tokyo, Japan) to evaluate root surface morphologies. Transmission electron microscopy (TEM) slices were prepared as described by Kong et al. [233], with minor modifications. TEM (Hitachi HT7700) was used to observe ultrathin sections at an acceleration voltage of 80 kV. For each treatment, at least three plants were evaluated, and plant images were representatively

selected for every treatment.

Autophagic levels

Root tips were used to determine autophagic levels by LysoTracker Red staining [231]. Root tips infiltrated with 100 μm E - 64d (Sigma, St. Louis, MO, USA) were stained with 2 μm Lyso - Tracker Red DND - 99 (Invitrogen, USA) as described by Liu et al. [228]. Fluorescence signals were observed by confocal microscopy (Carl Zeiss LSM780, Germany).

Subcellular grading

Based on subcellular grading methods by Weigel et al. [233], cells from fresh root samples were divided into 3 components: soluble fractions, cell walls and organelles. Fresh roots (0.5 g) were ground using a quartz mortar with a precooled homogenate solution (8 ml), supplemented with sucrose (0.25 mm), Tris - HCl buffer solution (50 mm; pH 7.5) and dithiothreitol (1 mm). First, the mixtures were centrifuged for 10 min at 2000 g and 4 ℃. The obtained precipitate was the cell wall. The supernatants were centrifuged at 4 ℃ for 45 min at 12000 g. The bottom fragments comprised organelle components, while the upper supernatant comprised the soluble fraction, which included inorganic ions and macromolecule organic matter from the vacuole and cytoplasm. Finally, cell wall and soluble fractions were dried at 50 - 60 ℃, after which they were weighed. The concentrations of Cd ions in the three subcellular components were measured by ICP - MS.

Chlorophyll content

Chlorophyll concentrations (mg / g) were assessed as described by Khan et al. [234]. Briefly, fresh leaves of the transgenic plant (0.1 g) were extracted using 5 ml of acetone (80% (v / v)). The extract was treated in the dark for two days then its absorbance was spectrophotometrically determined at 645 nm and 663 nm. (TU - 1810, Puxi, China)

Cadmium content

Transgenic wheat tissues were washed with deionized water and dried at 80 ℃ for 72 h, then weighed to 0.5 g. All samples were digested with 5 ml HNO_3 and 2 ml H_2O_2 by a microwave digestion instrument at a temperature variation regime of 160, 110, 160 ℃ and for 30, 30, and 30 min, respectively (SONNEN, X42A, China). The Cd concentrations were measured by Inductively coupled plasma mass spectrometry.

Conclusions to Chapter 2.

1. Experiment 1 was carried out in conditions favorable for winter wheat growing. The conditions of the vegetation period during the research were generally close to those typical for the zone, both in terms of rainfall and average daily temperature. As initial material, 41 samples of winter wheat from different breeding establishments were used. To determine the selection value of the initial and hybrid material correlation, dispersion, cluster analysis were used. In experiment research methods common for wheat crop were used.

2. In experiment 2, molecular biology approach was used to study the molecular

mechanism of cadmium resistance in winter wheat. A novel GOT / SFT2 protein family member (SFT2L) was functionally characterized in wheat. Method of gene discovery and functional validation in *TaSFT2 - like* (*TaSFT2L*) homologous cloning and expressions analysis was used. We assessed the correlations between *TaSFT2L* gene expressions in wheat and Cd accumulation. It is necessary to describe the method of gene discovery and functional validation in order to confirm and understand the reliability of subsequent experiments.

CHAPTER 3

COLLECTION STUDY AND OBTAINING THE SOURCE MATERIAL OF WINTER WHEAT WITH LOW ABILITY OF CADMIUM ACCUMULATION

3.1. Structure characteristic of the variety collection of winter wheat

The formation of a working collection was carried out on the basis of winter wheat varieties grown in the North - Eastern Forest - Steppe of Ukraine. It was based on passport data and the findings of a study conducted at the Institute of Agriculture of Ukraine's North-East. There were selected 40 varieties representing 7 major breeding centers of winter wheat in Ukraine, namely: Selection Genetic Institute National Center for Seed Research and Variety Studies (Odesa); Plant Production Institute named after Yuriev (Kharkiv); Institute of Agriculture (Kyiv); Bila Tserkva experimental breeding station (Bila Tserkva); Institute of Irrigated Agriculture (Kherson), Myronivka Institute of Wheat named after Remeslo, (Myronivka), Ivanivska experimental breeding station (Ivanivka village, Sumy region) (Figure 3.1).

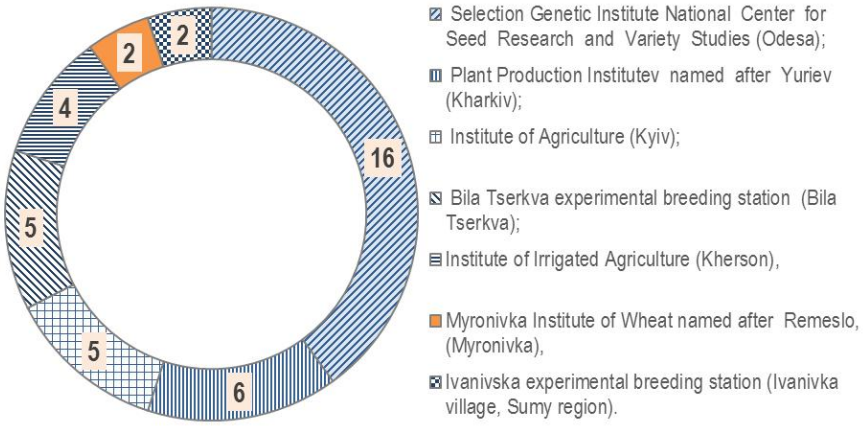


Figure 3.1. The structure of the collection of winter wheat by the originator.

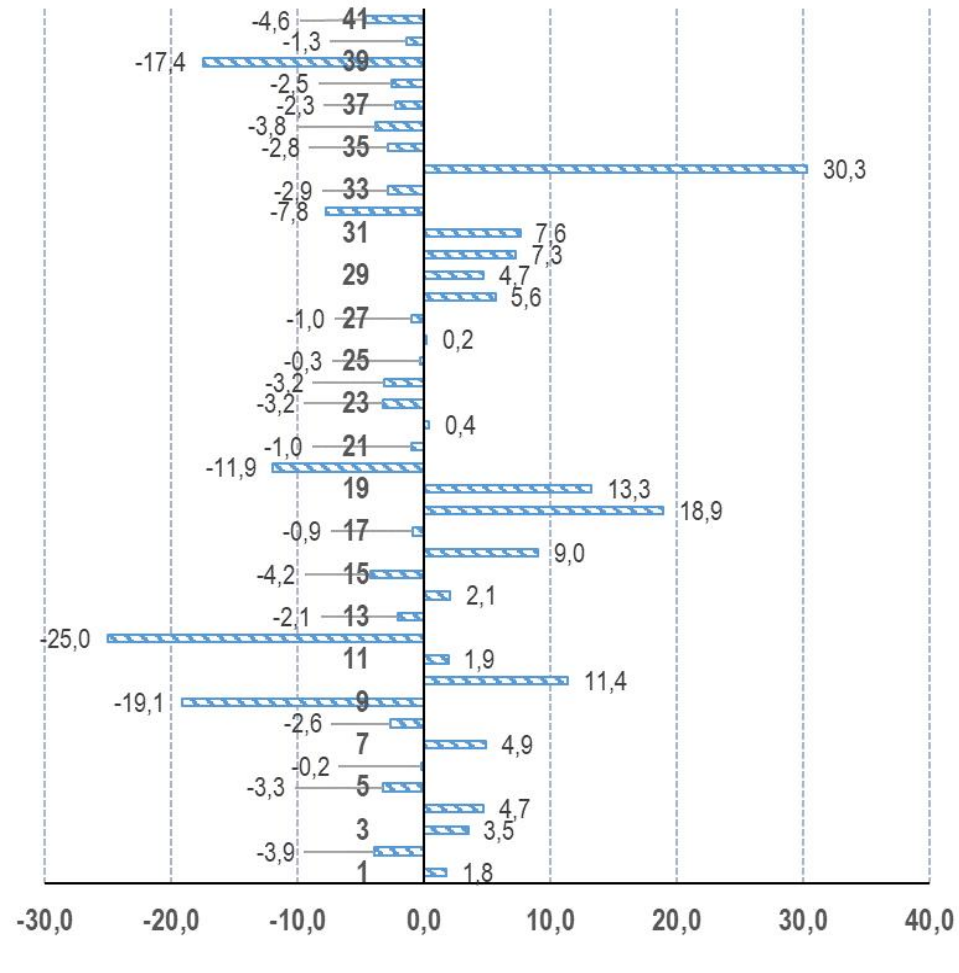
The largest group of varieties was created at Selection - Genetic Institute –National Center for Seed Research and Variety Studies - 16 or 40%. Varieties of the Plant Production Institute accounted in the collection as 5 (12,5%). Six (6) (15%) varieties were created by Bila Tserkva experimental - breeding station, 5 (12,5%) – by Institute of Agriculture, 4 (10%) – by Institute of Irrigated Agriculture, 2 (5%) – by Myronivskyi Institute of Wheat named after Remeslo, 2 (5%) – by Ivanivska experimental breeding station.

An important characteristic of varieties is the range of their variability in the main indicators of vegetative and generative development, especially plant height, leaf surface area, grain weight from the ear and yield.

Plant height is an important agronomic characteristic connected with development, growth and grain yield formation in wheat. Plant height is associated with a lodging reduction, growth in the grain number per ear and an improvement in the yield index and thus an increase in grain yield and quality. Understanding how different traits impact to plant height can help breeders select related traits more effectively.

The structure of the collection by trait of plant height is presented in Figure 3.2. (2018 - 2021). It was fixed that varietal characteristics of winter wheat plants affected plant height.

The average value of the indicator was 89.1 cm. The highest values of the average height (more than 1.0 m) were observed in the varieties of Okhtyrchanka juvilejna, Pylypivka odes'ka and Zaotar.



Average for the collection is 89.1 cm.

Figure. 3.2. The collection structure of winter wheat varieties on the base of stem height.

The maximum value of the average height was observed in the Rusyava variety - 119.4 cm.

Svitanok myronivskyi had the lowest indicator value of 64.1 cm. Statistically significant lower values of this indicator (compared to the average for the collection) were characteristic of Rozkwit, Krugozir and Hurt varieties.

The photosynthetic surface of both the whole plant and its individual organs

is of great importance in the productivity of winter wheat. When creating a hybrid source material, along with the elements of productivity, the formation of the leaf surface is very important. The structure of the collection according to the index of the crop leaf surface (LAI) is showed in Figure 3.3.

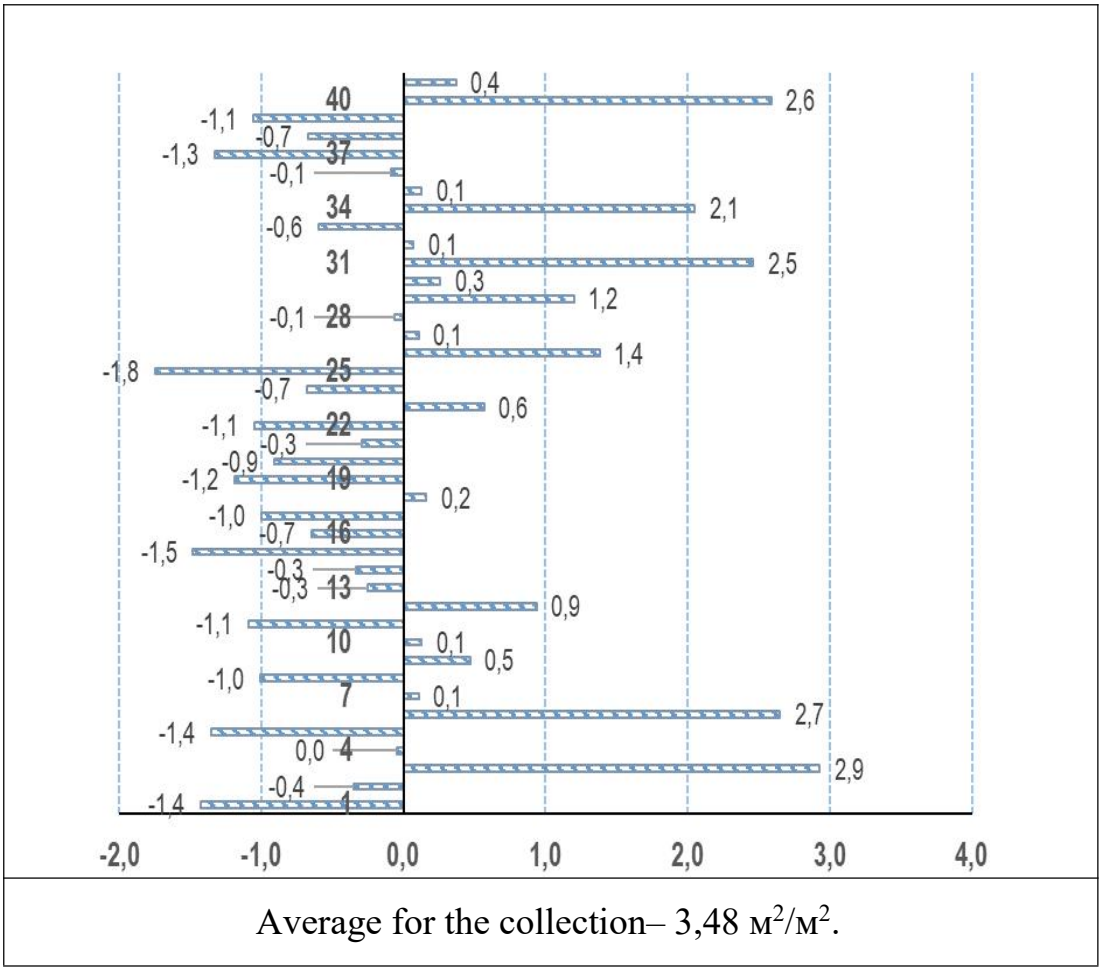


Figure 3.3. The collection structure of winter wheat varieties according to the index of the crop leaf surface.

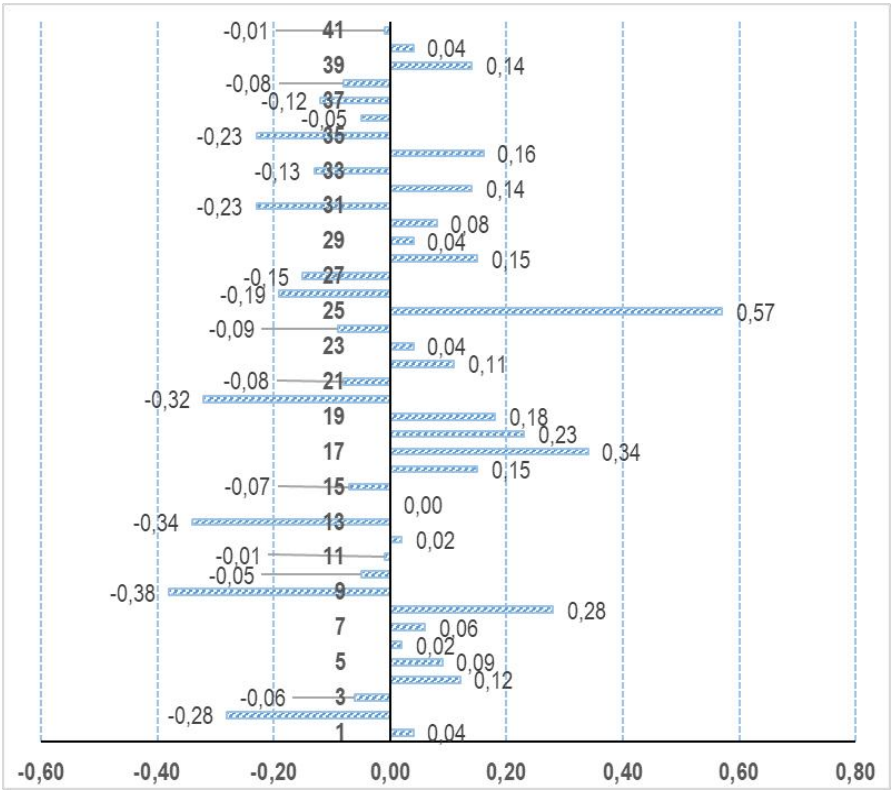
In general, this parameter characterizes the ability of the crop to form and maintain optimal leaf surface area per unit area. It is currently believed that for most varieties of winter wheat focused on the Forest - Steppe zone, the optimal value of the LAI is 3 - 4 m^2 / m^2. The formation of higher values of the index, as a rule, requires a change in the relationship between groups of chlorophyll in the direction

of shade - tolerant chlorophyll "b".

In other cases, the increase in the values of the indicator (due to increasing the density of crops or doses of mineral fertilizers) is accompanied by deterioration of the phytosanitary condition of crops and reduced efficiency of photosynthesis.

The average value of the index was 3.48 m^2 / m^2, varying from 1.68 in the variety Zdobna to 6.38 in the variety Alliance.

One of the main selection - controlled parameter of winter wheat is the mass of seeds per ear (Figure 3.4); it combines the number of seeds per ear and 1000 seeds weight.



Average for collection– 1,21 g.

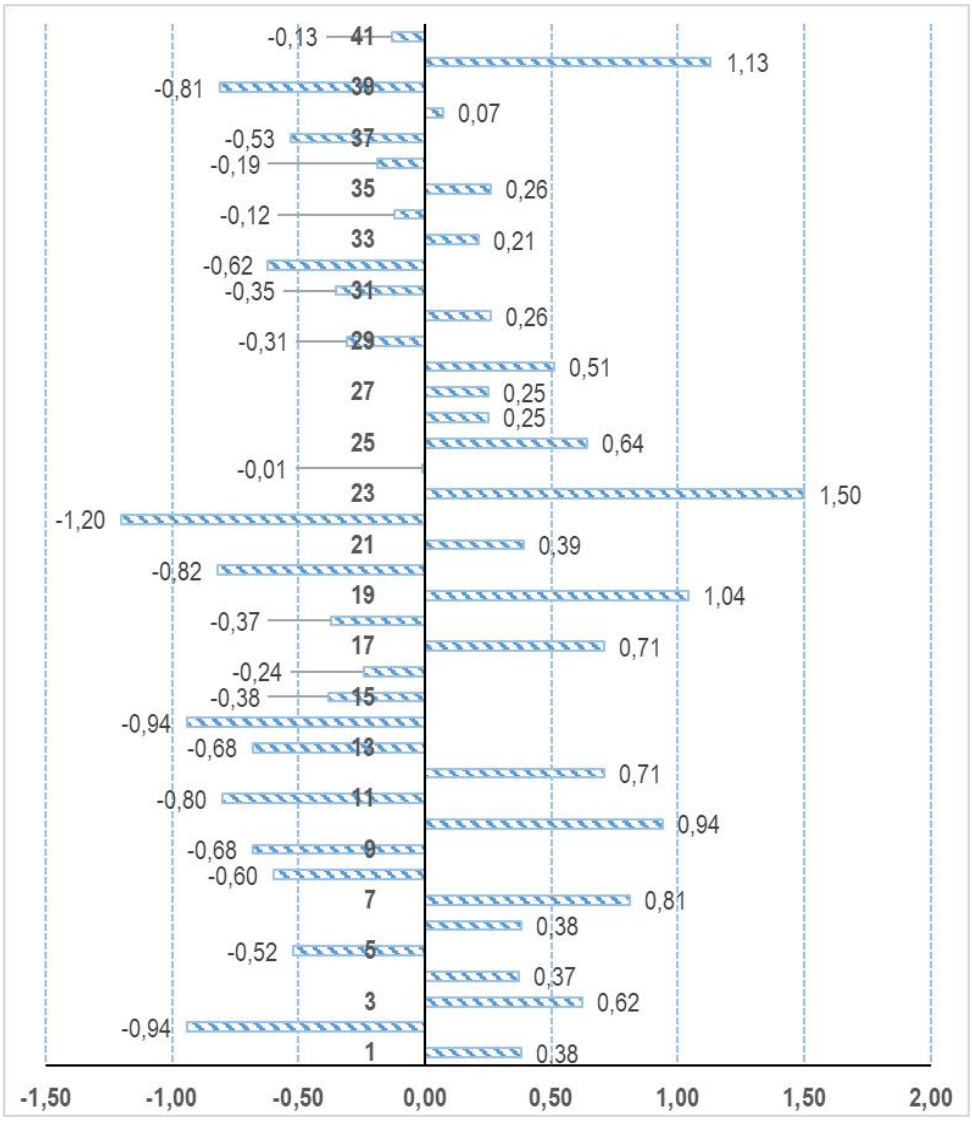
Figure 3.4. The collection structure of winter wheat varieties by grain weight per ear, g.

Taking into account the insignificant level of phenotypic variation of both traits due to their rigid genetic fixation, the characteristics of the collection of varieties, generally involve the identification of groups with different schemes for realizing plant genetic potential. The maximum values of the ear productivity index for the collection were observed in the varieties Zdobna, Oberig myronivskyi, and Osayna at the level of 1.21 g.

In all cases, high values of productivity were provided above the average values of both components. On the contrary, the minimum values were observed in the varieties Hurt, Melody odes'ka and Rozkvit due to a significant decrease in one of the structural indicators of productivity.

Grain yield is a complex trait and highly influenced by many genetic factors and environmental fluctuations. A successful breeding program is dependent on knowledge of genetic variability and the relationship of morpho-agronomic traits with grain yield.

A generalizing feature of the breeding value of variety is productivity and yield capasity. Analysis of Figure 3.5 shows that the average yield of the collection for the research years is 6.54 t / ha, varying range from 5.34 for the Klad to 8.04 t / ha for the Khvala variety.



Average for collection– 6.54 t / ha.

Figure 3.5. The collection structure of winter wheat varieties on the base of yield.

Significantly higher (average in the collection) yields were observed in varieties of Alliance, Vidrada, Okhtyrchanka juvileina, Svitanok myronivskyi, Oberig myronivskyi, Pylypivka ode'ska, Zdobna, Zorepad and Kraevyd.

3.2. Feaures of the variety collection of winter wheat based on the ability of Cd accumulation.

The previous stage of growing winter wheat in the conditions of the analytical background with a concentration of Cd in the soil of 1.0 g / kg allowed to evaluate the varieties for their ability to accumulate this toxic element in the vegetative organs. Data on the cadmium content in above ground phytomass are presented in Figure 3.6.

The average Cd concentration for 40 varieties was 1.40 mg / kg. The indicator value varied in the range from 2.02 in the variety of Duma odes'ka to 0.91 mg / kg in the variety of Oktava odes'ka. In addition to the last variety, the group with the minimum level of Cd accumulation (less than 1.0 mg / kg) included: Svitanok myronivskyi, Melody odes'ka and Kubok.

The following varieties had a statistically lower level of Cd content as well (compared to the average for the collection): Okhtyrchanka juvileina, Zorepad Bila Tserkva, Ovidiy, Shchedra nyva, Slaven.

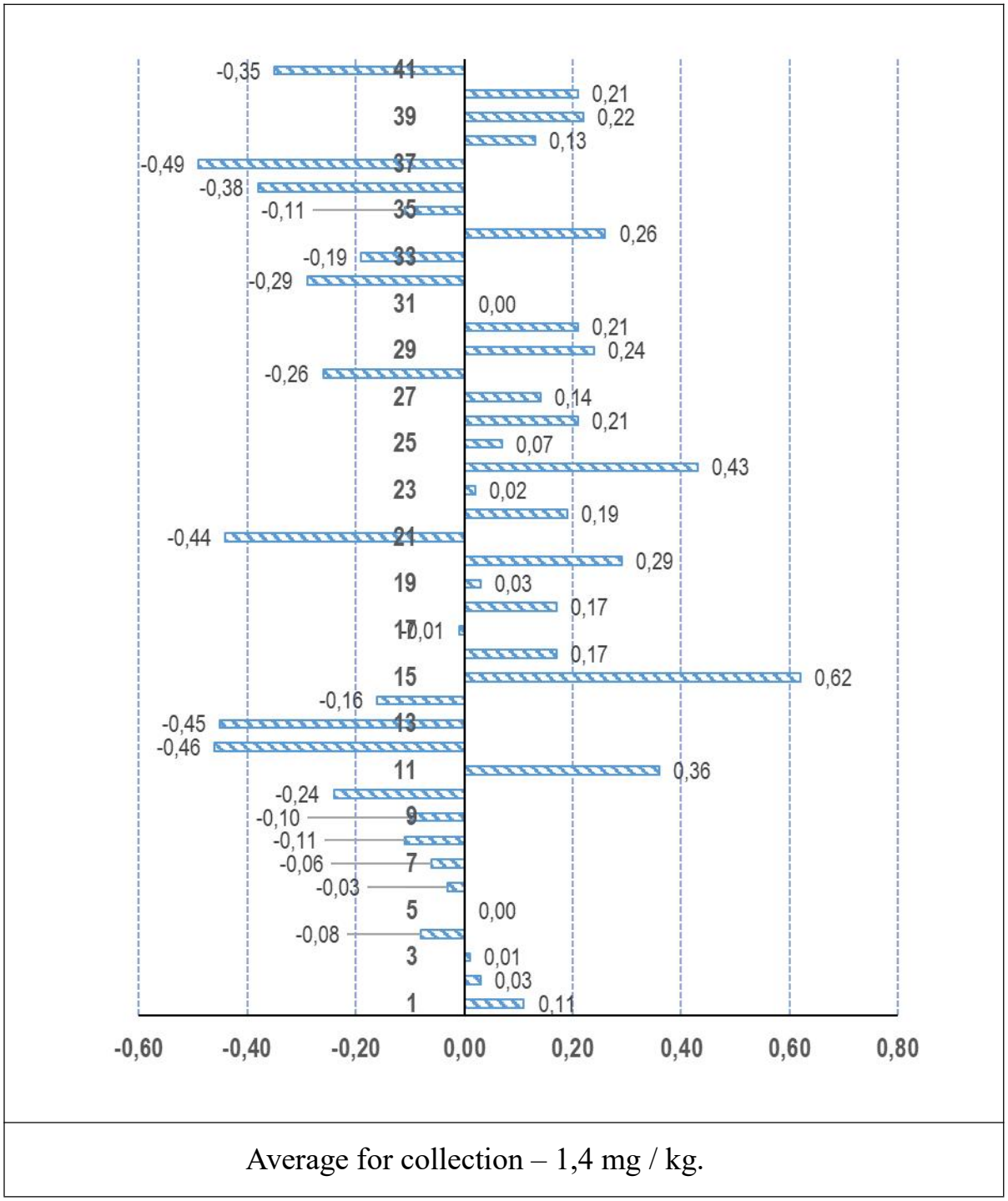


Figure 3.6. The collection structure of winter wheat varieties based on the ability of Cd accumulation on the analytical background.

The final stage in the formation of the working collection was the creation of groups based on several selectively valuable parameters and assessment of the level

of intragroup correlations for inter - varietal crossings.

According to the peculiarities of spatial distribution of varieties, depending on the values of Cd content and the main parameters of plant productivity, two groups of varieties were formed: those with low Cd content (less than 1.2 mg / kg) and those with high Cd content (more than 1.6 mg / kg) (Figure 3.7.).

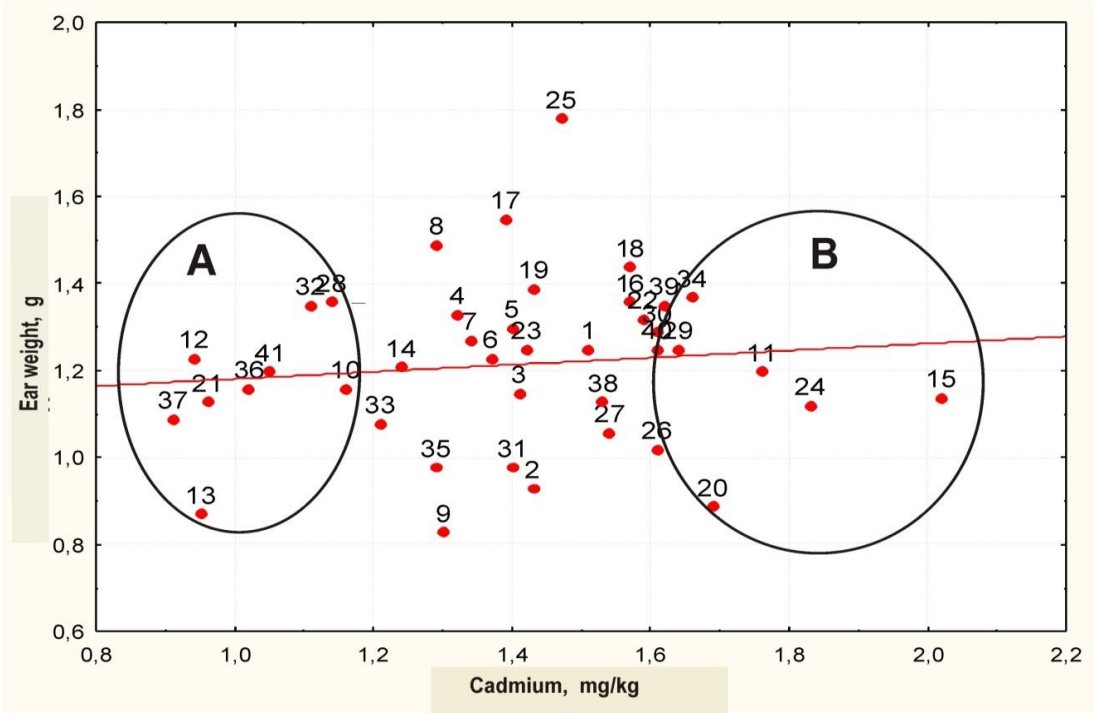


Figure 3.7. Spatial placement of varieties based on ear weight (g) and Cd content mg / kg: A - group of varieties with low Cd accumulation; B - variety group with a high level of Cd accumulation

A variety group with Cd values greater than 1.6 mg / kg was also selected. The first group "A" includes varieties of Okhtyrchanka juvileina, Svitanok myronivskyi, Melody odes'ka, Kubok, Zorepad, Ovidiy, Shchedra nyva, Oktava odes'ka and Slaven.

Such varieties as Sich, Duma odes'ka, Rozkvit, Kantata odes'ka and Rusyava

were included in group "B" with the maximum values of Cd. It should be noted that some high - yielding varieties, namely 25, 8, 17, 18 and 19 were characterized by average Cd content (1.3 - 1.5 mg / kg) and were not included in the crossing groups. In general, the groups were characterized by the following values for the main selectively valuable traits (table 3.2.1., Appendix C3-C4)

Table 3.2.1.

Traits of plant vegetative and generative development of winter wheat varieties in groups with different cadmium content

Trait	X ± st.	Min	Max	Vk,%
A – group with Cd content < 1,2 mg / kg				
Cadmium content, mg / kg	1,03 ± 0,03	0,91	1,16	9,02
Plant height, cm	85,87 ± 3,32	64,14	100,56	11,62
Mass of stem, g	5,2 ± 0,25	4,23	6,19	14,65
Leaf area, cm^2	62,2 ± 4,6	40,3	80	22,41
LAI, m^2/m^2	3,42 ± 0,20	2,15	4,42	17,71
Chlorophyll content, mg/g	2,07 ± 0,01	2,03	2,13	1,44
Final density, pcs/m^2	567,88 ± 23,75	438,5	673,6	12,54
Productivity, t / ha	6,58 ± 0,20	5,86	7,48	9,24
Seed weight per ear, g	1,17 ± 0,04	0,87	1,36	12,47
Number of seeds per ear,	31,42 ± 0,81	27,63	35,4	7,75

pcs				
1000 seed weight, g	37,46 ± 1,76	29,8	48,95	14,11
LAR, m^2/kg	5,2 ± 0,27	3,57	6,09	15,87
B - group with Cd content > 1.6 mg / kg				
Cadmium content, mg / kg	1,79 ± 0,06	1,66	2,02	8,00
Plant height, cm	91,71 ± 7,26	77,22	119,41	17,72
Mass of stem, g	5,07 ± 1,01	3,55	9,09	44,85
Leaf area, cm^2	61,12 ± 15,2	40,44	120	56,51
LAI, m^2/m^2	3,05 ± 0,63	2,0	5,53	46,19
Chlorophyll content, mg/g	2,05 ± 0,01	2,04	2,06	0,4
Final density, p.s./m^2	542,6 ± 32,61	468,61	642,7	13,43
Productivity, t / ha	6,11 ± 0,17	5,72	6,53	6,14
Seed weight per ear, g	1,14 ± 0,07	0,89	1,37	15,09
Number of seeds per ear, pcs	32,45 ± 0,97	29,35	34,65	6,07
1000 seed weight, g	35,2 ± 1,88	30,49	41,65	12
LAR, m^2/kg	4,96 ± 0,93	3,24	8,61	42,26

The lack of correlation between indicators of Cd content and indicators of plant productivity made it possible to form variety groups close in terms of productivity. Thus, almost identical average values were noted for the seed weight trait, namely 1.17 + 0.04 for the group with a low Cd content and 1.14 + 0.07 for the group with a Cd content of more than 1.6 mg / kg.

Groups differed more significantly in terms of productivity and 1000 seed weight. The group of "low Cd varieties" had higher average values of these indicators and a greater range of their variability. Thus, the yield variation coefficient in the group of varieties with a low level of Cd accumulation was 9.24% against 6.14% for the opposite group.

Regarding the values of the coefficient of variation, the highest values of the indicator were observed in the group of varieties with a high content of Cd for the group of parameters of vegetative development, namely the stem weight - 44.85%, leaves area - 56.51% and LAI - 46.19%.

In general, the characteristics of the formed groups made it possible to consider the possibility of cross - breeding based solely on the indicator of Cd content with the prospect of obtaining intervarietal hybrids with high indicators of plant productivity.

An important stage preceding the cross - breeding was the estimation structure of correlations within the formed groups (Appendix C4).

The analysis of the correlation structure of "A" group indicates that a statistically reliable level of correlation of the Cd concentration trait occurs among the parameters of plant vegetative development: plant height (r=0.54) and stem weight (r=0.88). In addition, a high level of correlation was noted with the index of seed weight per ear (r=0.60) and 1000 seed weight (r=0.74). A separate trait group was made up of the parameters of the plant leaf development.

With the general similarity of the correlations structure, that is the presence of parameters block of vegetative development and productivity of plants and a block,

characterizing the state of leaf area development, group "B" had a number of specific correlations. First of all, this is the relationship between indicators of cadmium concentration and LAR (r= - 0.68).

The presence of the Cd concentration indicator in different correlation groups with both minimum (“A”) and maximum (“B”) accumulation of this heavy metal indicates that the accumulation level (minimum or maximum) is controlled by different genetic mechanisms.

3.3. Estimation of created hybryds of winter wheat as an initial material with low Cd uptake.

The seeds obtained as a result of crossing in 2019 were sown (in September) and grown during 2019 - 2020. After the rejection (April 2020) of vegetation and observations, 31 hybrid samples (which had 5 or more normally developed and tilled plants) were selected.

The list of hybrids and their numbering is presented in Appendix B.

3.3.1. Characteristics of intervarietal hybrids collection

The average values, limits of variation and coefficients of variation of the main economic and valuable characteristics of the obtained hybrids are presented in the Table 3.3.1.

Table 3.3.1

Characteristics of the collection of intervarietal hybrids according to the main economic and valuable characteristics (2020 - 2021)

	Average	Asymmetry	Min	Max	Kv,%
Cd content in grain, mg / kg	0,2542	1,349661	0,0400	0,9100	106,4743
Height, cm	72,1647	0,334143	56,1300	87,1800	11,4896
Length of ear, cm	8,6360	0,867641	7,1600	11,6500	11,3029
Grain numder per ear, pcs	31,5219	0,418811	22,3300	43,7300	15,6555
Grain weight per ear (g)	0,9783	0,024725	0,5198	1,4600	22,4661
1000 grain weight, g	31,9013	0,239661	19,2500	48,4000	29,0557
Estimated productive density, ear /m^2	410,2774	0,662096	324,6000	554,6000	14,2675
Estimated yield, t / ha	3,7798	0,587178	2,0827	6,0362	23,4584

The average Cd content in the obtained hybrids was 0.254 mg / kg, with a low of 0.04 and a high of 0.91. The coefficient of variation was 106.5%.

Rather high value of the asymmetry coefficient indicates a rightward shift of indicators, that is, there is a tendency towards indicators whose values are higher than the average.

From our point of view, such a high level of variation in the indicator of Cd content in seeds is explained by the crossing scheme, that is, crossing within groups

with the minimum and maximum concentration levels of this metal.

The level of variation of indicators in the collection of hybrids, close to the average, was noted for the 1000 seed weight indicator - 29.1%, for the estimated yield - 23.5% and for the mass of grains from the ear - 22.5%. The average values for these indicators were 31.9 g; 3.78 t / ha and 0.97 g, respectively.

The coefficient of variation of such indicators as plant height, ear length, number of grains per ear and productive density was in the range from 11 to 15%, which approximately corresponds to the level of variation of these indicators in the working collection of varieties.

Positive results regarding the characteristics of the obtained hybrids were determined based on the results of correlation analysis (Appendix C2).

An average and significantly significant level of correlation was determined between the indicator of Cd content in seeds and indicators of crop density (r= - 0.41) and estimated yield (r= - 0.38).

The negative value of the correlation traits indicates the possibility of improving grain quality parameters (Cd content) due to the selection of plants with a high level of winter resistance and an increased tillering coefficient (Table 3.3.2)

According to the requirements in force in Ukraine, the level of Cd content in wheat seeds should not exceed 0.1 mg / kg for food grain, 0.2 and 0.3 mg / kg for grain exported or used for feeding, respectively.

Table 3.3.2

Characteristics of intervarietal hybrids in groups with different cadmium content (2020 - 2021)

	Average	Asymmetry	Min	Max	Kv,%
Group of hybrids with cadmium content < 0.1					
Cd content in grain, mg / kg	0,06	0,237556	0,0400	0,0900	28,86430
Height, cm	71,9541	0,078460	56,1300	87,1800	14,14091
Length of ear, cm	8,3873	0,353344	7,2000	10,0400	10,42083
Grain numder per ear, pcs	32,2909	0,381204	24,0500	43,7300	18,84208
Grain weight per ear, (g)	1,0384	0,168692	0,6494	1,4600	24,21043
1000 grain weight, g	33,4655	0,285017	19,8000	48,4000	33,30117
Estimated productive density, spikelets /m^2	418,3091	0,093365	352,8000	482,3000	11,20134
Estimated yield, t / ha	4,1233	0,687653	2,7782	6,0362	27,18267
Group of hybrids with cadmium content > 0.6					
Cd content in grain, mg / kg	0,7567	0,16626	0,6200	0,9100	14,32362
Height, cm	74,0650	- 1,27947	65,3300	79,2200	6,57049
Length of ear, cm	9,0975	1,49044	7,5600	11,6500	15,04519

Grain numder per ear, pcs	32,4417	0,31370	26,7900	39,5000	16,33804
Grain weight per ear, (g)	0,8711	- 0,57111	0,5198	1,1500	25,28954
1000 grain weight, g	27,2467	0,81394	19,2500	38,0700	29,39880
Estimated productive density, spikelets /m^2	370,9500	0,15065	324,6000	421,8000	9,49051
Estimated yield, t / ha	3,0523	0,66063	2,0827	4,3143	26,42563

In the hybrid group with a minimum level of Cd accumulation, the indicator of the metal concentration was in the range from 0.04 to 0.1 mg / kg. This group included 11 hybrids.

Indicators of controlled parameters, which were average for the group, were 71.9 cm for plant height, 8.4 cm for ear length. The indicator values of grain number per ear, the weight of 1000 seeds and the estimated yield were: 32.3 pcs., 33.5 g and 4.1 t / ha, respectively. All values were characterized by a right - sided type of distribution.

In contrast, the group of hybrids with a high level of Cd content in grain (> 0.6), which was obtained in combinations with the crossing of Duma odes'ka, Sich, Rozkvit, Kantata odes'ka and Rusyava varieties (6 hybrids), had slightly lower group parameters of some controlled indicators of the yield structure. Thus, the values of grain mass indicators from ear and 1000 seed weight were 16.5 and 18.6% lower and amounted to 0.87 and 27.24 g, respectively.

In addition, the distribution of values according to plant height and grain weight from the ear was characterized as left - sided, which indicates the dominance in the samples of low - height plants with a small ear weight. A narrowing of the range of indicators and a decrease in the values of the coefficient of variation were characteristic of most of this group.

However, in our opinion, this factor can be explained by the smaller number of the group. In addition, the distribution of values according to plant height and grain mass from the ear was characterized as left - sided, which indicates the dominance in the samples of low - growing plants with a small mass of the ear. The majority of this group was characterized by a narrowing of the range of indicators and a decrease in the values of the coefficient of variation. However, in our opinion, this fact can be explained by the smaller number of the group.

The groups differed significantly in the structure of correlations. Thus, a characteristic feature of hybrids with minimal Cd content in seeds were inverse and statistically significant correlations between the values of Cd content and indicators of the seed weight from the ear (r= - 0.70), 1000 seed weight (r= - 0.78) and the indicator of estimated yield (r= - 0.74).

In the group of hybrids with a high level of Cd concentration in seeds, a reliable level of correlation between the this indicator values and the controlled traits was not found.

The revealed dependences allow us to assume the "technological nature" of the formation of a group with a low level of Cd concentration. This supposition is supported by the high level of correlation of the trait with direct indicators of plant

productivity. An additional factor should be considered the peculiarities of the field experiment, namely the use of high doses of mineral fertilizers. This measure ensured the detection of genotypes capable of efficient use of environmental resources, primarily due to the increase in the values of generative traits.

Under these conditions, the decrease in the concentration of Cd in seeds occurred due to an increase in the supply of organic compounds.

The testing results of deveoped hybrids made it possible to identify samples of different genetic origin, suitable for futher breeding work to create new source material.

Within the groups identified by the content of Cd in the seeds, samples were taken according to the estimated yield of more than 3.0 t / ha

Thus, six samples were selected for winter wheat breeding programs with a low level of Cd accumulation.

Two samples were selected for breeding programs to study the characteristics of the manifestation and inheritance of the trait of low resistance to Cd accumulation.

Data on the dynamics of Cd content in wheat seeds are presented in Table 3.3.3.

The weather conditions of the 2019 - 2020 growing season were quite favorable for vegetation and the formation of the winter wheat yield. The average crop yield this year was 5.38 t / ha.

Table 3.3.3

Cadmium content in seeds of intervarietal hybrids of winter wheat, mg / kg

Breeding sample	Origin	year				X cp
		2020		2021		
		X	± standart	X	± до standart	
Relative standard		0,071		0,074		0,072
19/12	Melody odes'ka x Svitanok myronivskyi	0,032	0,039	0,05	0,024	0,041
19/13	Melody odes'ka x Shchedra nyva	0,034	0,037	0,051	0,023	0,043
19/26	Kubok x Svitanok myronivskyi	0,028	0,043	0,059	0,015	0,044
19/40	Shchedra nyva x Kubok	0,054	0,017	0,049	0,025	0,052
19/33	Zorepad x Okhtyrchanka juvileina	0,062	0,009	0,061	0,013	0,062
19/39	Shchedra nyva x Okhtyrchanka juvileina	0,048	0,023	0,08	0,006	0,064

The growing season of 2020 - 2021 was characterized by high temperatures and low precipitation during the period of grain formation and maturing. As a result, the average yield was 4.88 t / ha. The average values of Cd content in the seeds of

conventiona (relative) l standard varieties for these years were 0.071 and 0.074 mg / kg, respectively.

In 2020, a year with favorable weather conditions, all isolated samples had a lower Cd content compared to the conventional standard.

The maximum exceedance of the standard indicators - + 0.043 mg / kg - was noted in sample 19/26. By absolute value (in increasing order), the samples were arranged in the following order: 19/26; 19/12; 19/13; 10/39; 19/40 and 19/33.

A rather significant decrease in the average yield in 2021 was accompanied by some growth in Cd content indicators and a change in the rating of varieties. Minimum values of the Cd content index were noted in 19/40; 19/12 and 19/13, samples, namely 0.049; 0.05 and 0.051 mg / kg, respectively. The last places in the rating were taken by samples 19/39 and 19/33 with participation in the hybridization of the Okhtyrchanka juvileina variety. Their Cd content was 0.080 and 0.061 mg / kg.

On average, over 2 years of observation, the best (minimum) result in terms of Cd accumulation was demonstrated by samples 19/12 and 19/13 obtained in the hybrid combinations of Melody odes'ka x Svitanok myronivskyi and Melody odes'ka x Shchedra nyva.

3.3.2. The structure of plant productivity of intervarietal hybrids

An important characteristic of the source material is high and stable indicators for the main economic and valuable characteristics. Provided there is a sufficient level of variation, this provides the possibility of effective selection based on a

minimum number of traits, which significantly reduces the cost of the breeding program and speeds up its execution time.

As previously stated, significant differences in weather conditions reduced some of the structural indicators of plant productivity in the varieties used as a conditional standard. This is especially evident for the 1000 seed weight indicator, where the difference between 2020 and 2021 compared to the conditional standard indicator was 3.7g or 7.3%. The difference in seed number per ear indicators was less significant, amounting to about 5% (Table 3.3.4.)

Table 3.3.4.

The structure of plant productivity of intervarietal hybrids with low cadmium content

sample	Origin	Seed number per ear, pcs			1000 seed weight, g		
		2020	2021	Xav.	2020	2021	Xav.
Relative standard		37,5	35,8	35,5	47,5	43,8	44,6
19/12	Melody odes'ka x Svitanok myronivskyi	28,3	25,3	26,8	37,8	33,1	35,45
19/13	Melody odes'ka x Shchedra nyva	31,2	28,84	30,02	46,9	44,04	45,47
19/26	Kubok x Svitanok myronivskyi	29,8	30,54	30,17	52,4*	44,4	48,4
19/40	Shchedra nyva x Kubok	25,6	22,5	24,05	51*	43,8	47,4
19/33	Zorepad x	42,9*	44,56*	43,73	29,8	25,08	27,44

	Okhtyrchanka juvileina						
19/39	Shchedra nyva x Okhtyrchanka juvileina	27,8	21,26	24,53	47,8	41,88	44,84
LSD $_{0,05}$		3,04	3,21		1,14	1,42	

According to the indicator of the average seed number per ear, only one breeding sample, namely 19/33, statistically significantly exceeded the indicators of the conditional standard in both years of research. The best results were noted for the 1000 seed weight indicator.

In 2020, two samples, namely 19/26 and 19/40 exceeded the conditional standard at a reliable level. These samples and sample 19/13 had an indicator value close to the standard in 2021.

Ambiguous results for interpretation were obtained regarding the calculated indicators of the number of productive stems and the hybrid yield compared to the conditional standard (Table. 3.3.5.)

In both years of research, the conditional standard varieties had lower or the same indicators of the density of productive stems as the created hybrids. Thus, with an average number of stems per m^2 in intervarietal hybrids of 386.52 pcs/m^2, the range of indicators varied from 398.8 in sample 19/33 to 480.1 in sample 19/12. Intervarietal hybrids exhibited higher levels of productive tillering with close indicators of field germination and indicators of crop thinning in the winter.

Table 3.3.5.

Yield and yield structure of intervarietal hybrids with low cadmium content

Breeding sample	Origin	Estimated density of productive stems*, pcs/m^2			Estimated yield, t / ha		
		2020	2021	Xcp	2020	2021	Xcp
Relative standard		395,56	377,50	386,528	7,12	6,04	6,58
19/12	Melody odes'ka x Svitanok myronivskyi	489,2	471	480,1	5,38	3,77	4,57
19/13	Melody odes'ka x Shchedra nyva	470	460,2	465,1	7,05	5,98	6,51
19/26	Kubok x Svitanok myronivskyi	450,6	402,8	435,2	7,21	5,64	6,42
19/40	Shchedra nyva x Kubok	410,3	392,7	401,5	5,33	3,93	4,63
19/33	Zorepad x Okhtyrchanka juvileina	362,6	398,8	380,7	4,71	4,39	4,55
19/39	Shchedra nyva x Okhtyrchanka juvileina	402,6	418,6	410,6	5,23	3,77	4,5
HIP $_{0,05}$		23,4	21,9		0,48	0,42	

* - for sowing rate of 67 grains per linear meter.

In two years of research, they formed several tiers of productive stems with different levels of ear development, which significantly reduced the average grain weight per one ear.

In the experiment with intervarietal hybrids testing, the average seed weight of the standard was 1.6 g (1.8 g in 2020 and 1.6 g in 2021). Hybrid average values ranged from 1.0 to 1.5 g in samples 19/12 and 19/26, respectively. Under these conditions, only two among six selected hybrids, namely 9/13 and 9/26, had statistically the same estimated yield indicators as the conditional standard **(**Appendix C3). Undoubtedly, samples with extreme values of Cd accumulation in seeds are of scientific and breeding interest. Thus, samples 9/11 and 9/18, created in combinations of Sich and Duma odesk'a varieties with Okhtyrchanka juvileina variety had these indicators. The Cd content in these samples is two times higher compared to the top layer of 0.22 mg / kg. Characteristics of hybrids with the maximum level of Cd accumulation based on the main selectively controlled traits (Table 3.2.6.)

Table 3.3.6.

Characteristics of intervarietal hybrids with maximum cadmium content

(2020 - 2021)

№	Origin	Cd content in grain, mg / kg	Plant height, cm	Seed number per ear, pcs	Seed weight per ear, g	1000 seed weight, g	Estimated density of pro - ductive stems *, pcs./m^2	Estimat ed yield, t / ha
19/11	Sich x Okhtyrchanka juvileina	0,82	76,10	26,79	1,02	38,07	376,7	3,65
19/18	Duma odes'ka x Okhtyrchanka juvileina	0,91	72,93	31,34	1,15	36,70	394,9	4,31

* - for sowing rate of 67 grains per linear meter.

In general, the selected samples had indicators of vegetative development of plants that were close to the average for the hybrid collection and slightly lower values of productivity structure indicators.

Both samples were characterized by reduced indicators of productive bushiness, which with the minimum values of grain weight from the ear led to a low level of productivity of samples 9/11 and 9/18, namely 3.65 and 4.31 t / ha, respectively.

3.3.3. Distribution of the F1 hybrids by type of inheritance to cadmium accumulation

An important indicator of the efficiency of future research is the determination of the trait dominance level of the of resistance to Cd accumulation in F_1 intervarietal hybrids of winter wheat. The structure of the hybrid distribution in groups obtained from parents with the minimum and maximum content of Cd in seeds is presented in Table 3.2.7.

In total, 25 hybrids were obtained and tested in crosses within the group of varieties with minimal Cd content. The effect of heterosis or increased tolerance to the accumulation of Cd in the first generation was noted in 3 combinations: Melody odes'ka x Ovidyi, Kubok x Svitanok myronivskyi and Shchedra nyva x Okhtyrchanka juvileyna. the frequency of heterosis was 12%.

A rather significant share, namely 8%, was made up of hybrids with partial positive dominance. The range of fluctuations of the Cd content indicator for these groups of hybrids was 0.04 - 0.06 mg / kg.

Table 3.2.7

Structure of the F_1 hybrid distribution

Type of inheritance	Scheme of crossing combination	
	min	max
Heterosis	12	0
Depression	40	6
Partial positive dominance	8	0
Partial negative dominance	4	0
Intermediate inheritance	16	0
Dominance of parental form		
The best (min)	16	0
The worst	44	100

An intermediate type of inheritance was noted in 4 combinations or 16% of crosses. The largest share of combinations was with the participation of Shchedra nyva and Kubok varieties.

The largest proportion of crosses with the control of resistance to Cd accumulation, namely 24 and 40%, occurred by the type of partial negative dominance or depression. In the last case, the range of Cd content in seeds was from 0.1 to 0.42 mg / kg.

The greatest effect of depression was observed in combinations: Zorepad x Melody odes'ka, Melody odes'ka x Shchedra nyva and Ovidyi x Zorepad. In these crosses in F_1, the Cd content index exceeded the average value of the parent

varieties by 4.5 - 5.0 times.

As a result of crossing the group of varieties with the maximum Cd content, 6 hybrids were obtained. The maternal component in most combinations was the Duma odesk'a variety. Each one hybrid was formed by Sich and Kantata odesk'a varieties. The pollinators were varieties: Duma odes'ka, Sich, Roskvit, Kantata odes'ka, Rusyava.

Regardless of the direction of crosses, the first generation inherited the trait of resistance to Cd accumulation according to the type of depression. The range of values of this indicator in hybrids was 0.62 - 0.91. However, it was only 1.7 - 2.0 times higher than the average Cd content in parent varieties

A significant difference in the degree of phenotypic dominance of the indicator of Cd content in the seeds of winter wheat varieties confirms the assumption about the heterogeneity of the genetic mechanisms of the manifestation of the resistance trait.

On the contrary, the absence of phenotypic differentiation of the first generation indicates the universal dominant nature of the ability to accumulate high doses of Cd.

As summerize above mentioned excessive amounts of Cd in the environment have a negative effect on the growth and development and yield capacity of winter wheat plants. Wheat varieties vary extensively in their ability to uptake, accumulate, and to be toleratant to Cd [237].

Differences in Cd accumulation may depend on the adaptation of different genotypes to environmental and production conditions as well. [198]. Wheat

varieties with low - Cd accumulation are the most effective way to reduce risks that are related with food consumption.

Developing of wheat varieties with low Cd uptake and reduction Cd accumulation in grain can be realized by both conventional and modern breeding methods.

Conventional and molecular breeding approaches for wheat breeding have to a goal to minimize Cd uptake and its toxicity. Modern breeding tools also suggest a great potential for crop breeding programs that can be used alongside conventional selection in order to create varieties with low Cd utake [201].

The opportunities of traditional breeding is still an promosing approach to modifying the Cd uptake of wheat varieties. Using of heterosis also can open new approaches for decreasing winter wheat Cd uptake and adapting to Cd stresses [189, 237].

In conventional breeding, low - Cd wheat varieties are selected based on different traits (morphological, physiological, or biochemical) that are associated with Cd uptake. To improve the genetic background of wheat varieties with Cd tolerance, intra - specific crosses among superior individuals are usually developed, followed by selection in next generations. Breeding methods, such as mass selection, pure lines, and recurrent methods can be effectively used in the creation of low - Cd wheat varieties [236]. Conventional breeders are depended upon environmental variations and require a widespread location field trial, delaying the progress of variety development [194].

As a general 9–10 years of breeding process are required to get a variety right

from the pre - breeding phase up to commercial production [190].

Traditional breeding has been successfully utilized and considerable breeding progresses has been achieved in many parameters, such as yield, quality, and stress tolerance. Conventional methods for adaptation to abiotic stresses is challenging, as compared to breeding for other plant features. For each type of abiotic stresses there are various mechanisms of resistance, depending on the plant stress - adaptive nature [236].

Unfortually there are several negative aspects to low - Cd varieties of wheat breeding. Because it is time - consuming and the genetic improvement process is rather slow.

Regardless these difficulties plant breeders used to solve this problem (creation of low - Cd winter wheat varieties) by conventional breeding methods, i.e., introduction, selection, and hybridization.

As a result, several low - Cd wheat cultivars were developed owing to conventional breeding tools. For instance, three wheat varieties under four different Cd levels were studied (200). Their results listed several Cd - tolerant varieties, containing the lowest Cd content and relatively lower toxicity compared to other samples. Fifteen wheat varieties under Cd concentrations of 15, 30, and 45 μM were tested. The results showed that some of them had the lowest Cd content.

Though a large number of conventional studies were performed to develop out Cd - safe wheat varieties.

Conclusions to Chapter 3.

1. As a result of studying the collection samples of wheat (40 varieties) from different institution - originators, samples with valuable breeding characteristics were identified. Growth parameters such as height and leaf surface area were analyzed in the studied wheat varieties. These traits were related to productivity parameters such as 1000 seed weight, grain weight per ear, and yield.

2. The range of variation of the sign of Cd content in plants and grains of winter wheat varieties was determined: 0.91 - 2.02 and 0.06 - 2.56 mg / kg, respectively. Varieties had minimum indicators of Cd content in grain: Oktava odesk'a, Svitanok myronivskyi, Melody odes'ka, Kubok, Shchedra nyva. The maximum level of Cd content was noted in varieties of Rozkvit, Sich, Kantata odes'ka, Duma odes'ka.

3. In groups of varieties with different Cd content, differences in the structure of correlations between the main selection - controlled parameters were established. It was determined that the group of "low cadmium varieties" is characterized by the presence of direct correlations between the Cd content and parameters of vegetative development of plants, namely plant height (r=0.54) and stem weight (r=0.88). In addition, a reliable negative correlation was noted with the indicators of the seed weight per ear (r= - 0.60) and the 1000 seed weight (r= - 0.74).

4. In the variety group with a high content of Cd, a reliable level of correlation of metal content was with the LAR indicator (r= - 0.68) and the absence of statistically significant relationships with plant productivity indicators was noted.

Significant differences in the correlation structure of the Cd content trait in seeds between groups of varieties with high and low Cd content indicate a difference in the mechanisms of genetic control of this trait.

5. The distribution of inheritance frequencies of resistance trait to Cd accumulation in F_1 was calculated. After cross - breing low - Cd varieties, the frequency of inheritance according to the type of heterosis is 12%, incomplete positive dominance - 8%, intermediate inheritance - 16%, and according to the type of depression and incomplete negative dominance - 44%. Inheritance according to the type of heterosis was noted in the combinations: Melody odes'ka x Ovidyi, Kubok x Svitanok myronivskyi and Shchedra nyva x Okhtyrchanka juvileyna.

6. It was established that a characteristic feature of hybrids with minimal Cd content in seeds were inverse and statistically significant correlations between the values of Cd content and indicators of the seed weight per ear (r= - 0.70), 1000 seed weight (r= - 0.78) and estimated yield (r = - 0.74). In the group of hybrids with a high level of Cd concentration in seeds, a reliable level of correlation between the values of this indicator and the controlled traits was not found.

7. Six samples were allocated for selection work: 19/1, 19/13, 19/26, 19/40, 19/33, and 19/39, obtained in combinations of Melody odesk'a x Svitanok myronivskyi, Melody odesk'a x Shchedra nyva, Kubok x Svitanok myronivskyi, Shchedra nyva x Kubok, Zorepad x Okhtyrchanka juvileyna, Shchedra nyva x Okhtyrchanka juvileyna. The samples exceed the conventional standard in terms of Cd content in seeds, which is one of the crop structure indicators.

CHAPTER 4

THE DISCOVERY AND FUNCTIONAL ANALYSIS OF THE *TaSFT2L* GENE

4.1. The Cd stress resistant *AtSFT2L* knockdown mutant

The constructed lhRNA library from an *Arabidopsis* cDNA population can be used in future gene functional studies in plants. In this study, among the $CdCl_2$ - resistant transgenic lines, the HCA350 - 466 line [222, 231], which exhibited a resistance phenotype (Figure 4.1), was used for sequencing analyses of the silenced gene (*AtSFT2L*). The results showed that, in *Arabidopsis*, *AtSFT2L* has vital roles in Cd stress responses.

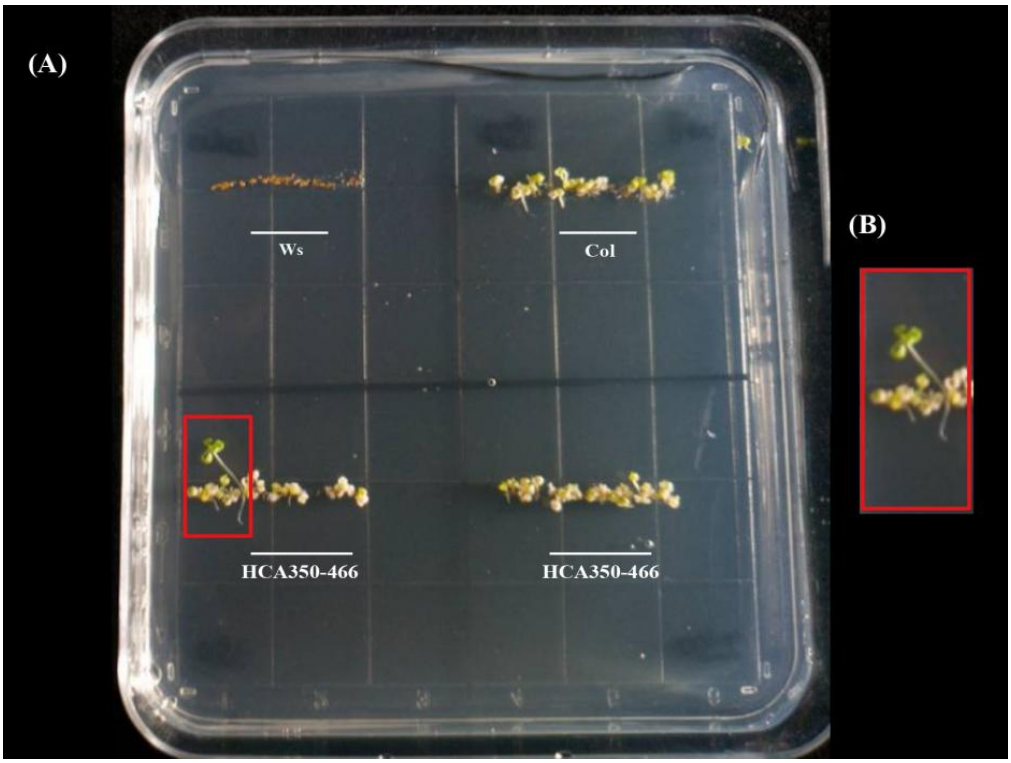


Figure 4.1. *AtSFT2* was screened from the *Arabidopsis* mutant library. Ws: Wassileskija type; Col: Columbia type; HCA350 - 466: *Arabidopsis* mutant lines. (B) is the enlarged visual field of (A).

4.2. Cloning and basic information analysis of *TaSFT2L*

4.2.1. Gene cloning of *TaSFT2L*

AtSFT2L was screened from the *Arabidopsis* mutant library using 0.1 mM $CdCl_2$. NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.blastn&PAGE_TYPE =BlastSearch &LINK_LOC =blasthome) was used to sequence *TaSFT2L* and homologous cloned from wheat Bainong 207. The corresponding gene had a 684 bp open reading frame (ORF) (Figure 4.2). The physical and chemical properties of TaSFT2L protein were analyzed by Protaparam, and the molecular formula was C_{2089} H_{3496} $N_{684}O_{889}S_{191}$, the relative molecular weight was 24.26 kDa, and the theoretical isoelectric point pI was 9.169.

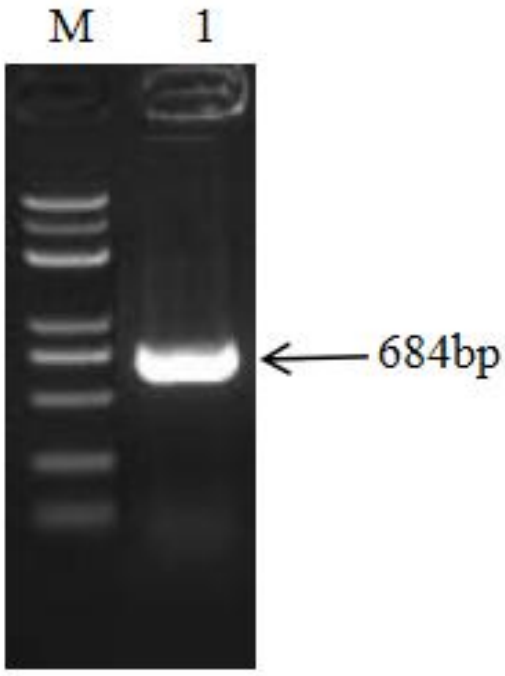


Figure 4.2. PCR amplification of *TaSFTL* gene. M: Standard 2000 + marker. 1: amplified band.

4.2.2. Basic information of *TaSFT2L* gene

SingalP4.1 analysis (Figure 4.3) showed that the sequence was a signal peptide that distinguished the transmembrane region. According to TMHMM Server v.2.0 online analysis, the TaSFT2L protein has four distinct transmembrane regions (Figure 4.4). Using Expasy online website (http://web.expasy.org/cgi - bin/protscale/protscale.pl?1), the hydrophilic / hydrophobic property of the amino acidsequence of this gene was analyzed (Figure 4.5.). The hydrophobic region encoded by TaSFT2L alternated with the hydrophilic region. Therefore, the TaSFT2L protein was predicted to be hydrophilic. In order to further study the evolutionary relationship of *TaSFT2L* gene in different species, the evolutionary tree of *TaSFT2L* gene in different organisms was constructed through Clustal W comparison in MEGA 5.0 and the Neighbor - joining method. A search of the Plant Transcription Factor Database (http://planttfdb_v3. cbi.pku.edu.cn/) showed that the predicted gene is a member of the wheat *GOT1/ TaSFT2L* vesicle transport protein family. To identify the subfamily of the *GOT1/ TaSFT2L* family, homology searches were conducted against protein sequences from other plants in NCBI. BLASTP analysis confirmed homology (more than 90% similarity) with other plant SFT2L proteins (Figure 4.6.). Accordingly, the gene was named *TaSFT2L*. Furthermore, the homology of *TaSFT2L* was most close to barley (Figure 4.7.).

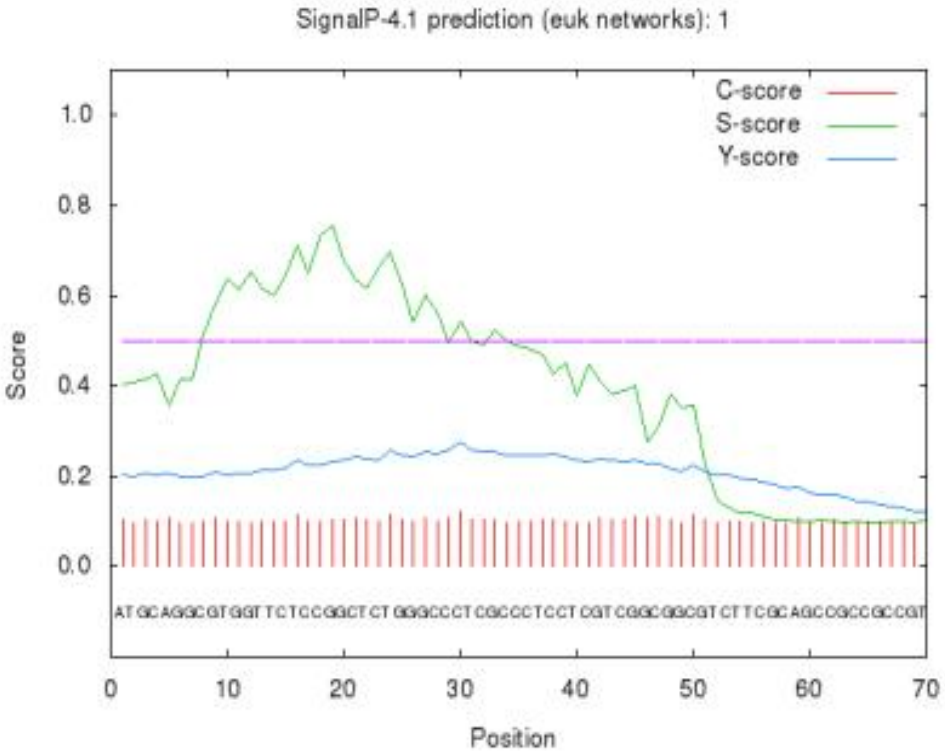


Figure 4.3. Signal peptide analysis of *TaSFT2L*.

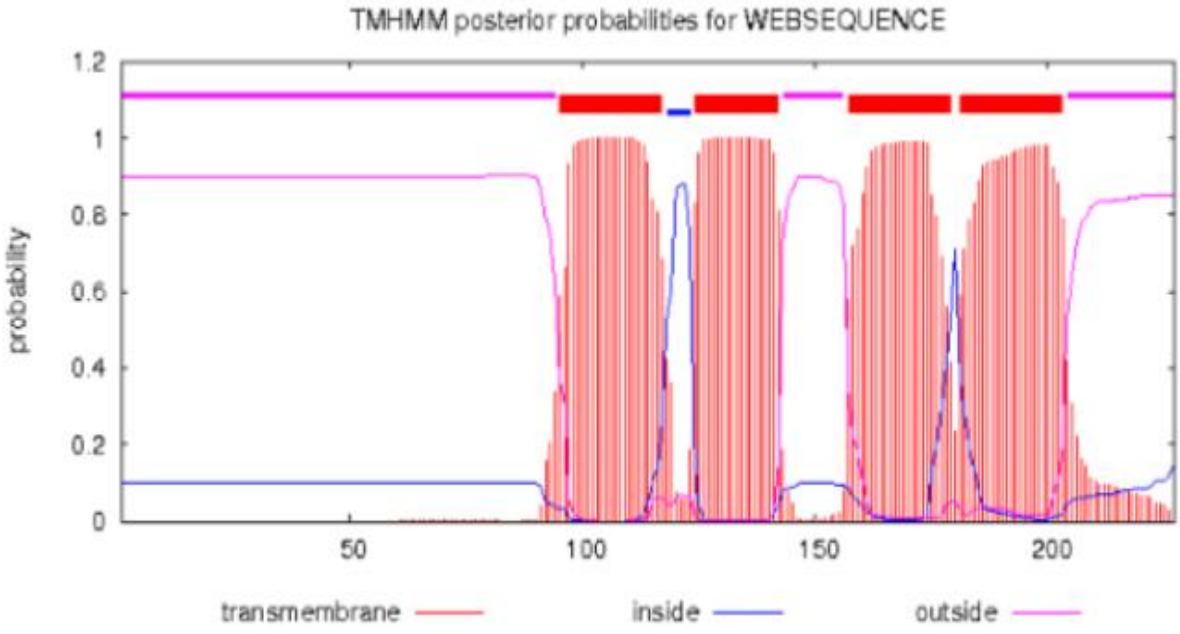


Figure 4.4. Analysis of transmembrane domain of *TaSFT2L*.

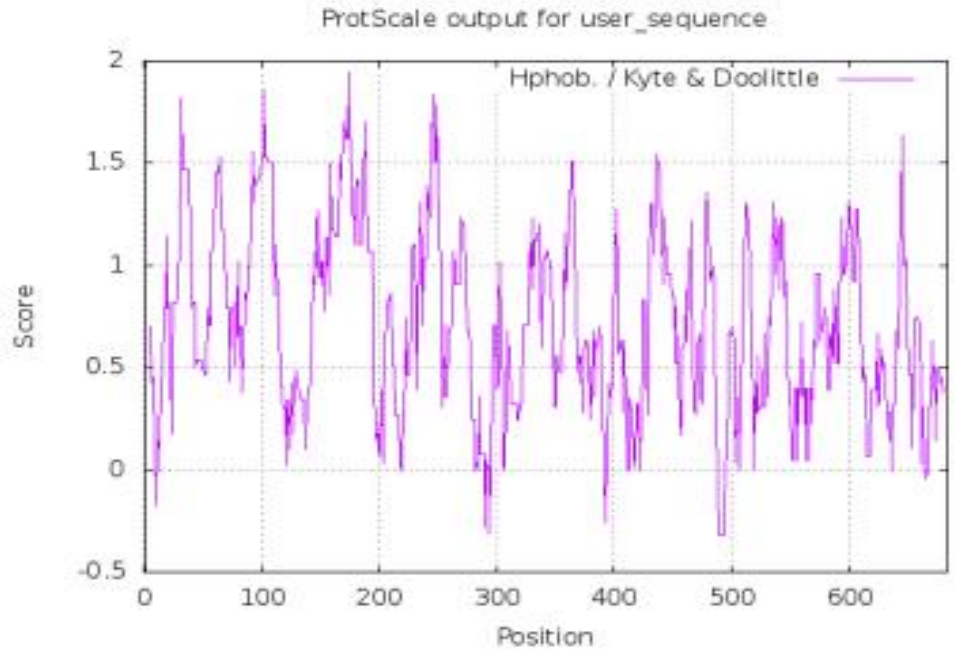


Figure 4.5. Hydrophilic and hydrophobic analysis of *TaSFT2L*.

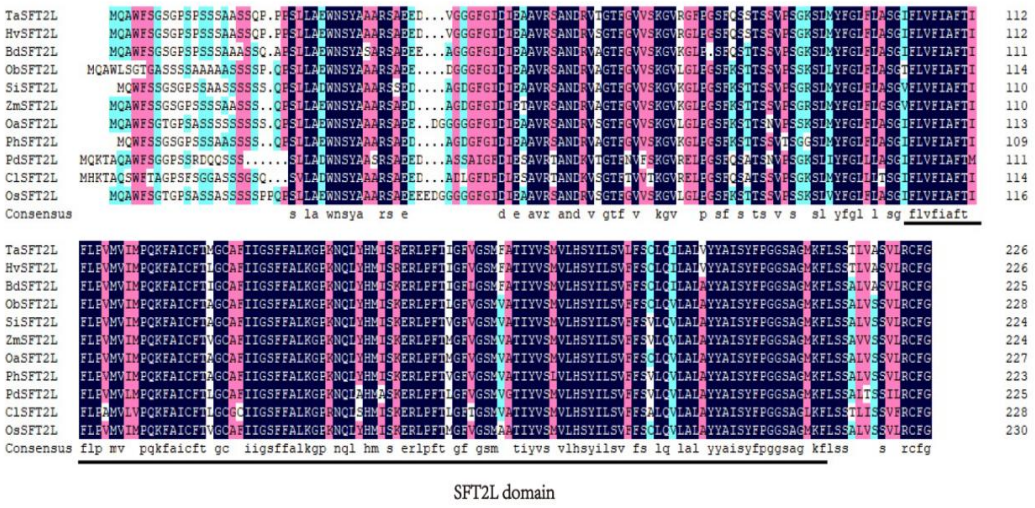


Figure 4.6. Domain alignment in TaSFT2L proteins. The black line indicates the conserved TaSFT2L domain and the consensus sequence is shown in black. Ta, Triticum aestivum; Hv, *Hordeum v ulgare*; Bd, *Brachypodium distachyon*; Ob, *Oryza brachyantha*; Oa, *Oryza alta*; Ph, *Panicum hallii*; cl, *Carex littledalei*; Os, *Oryza sativa.*

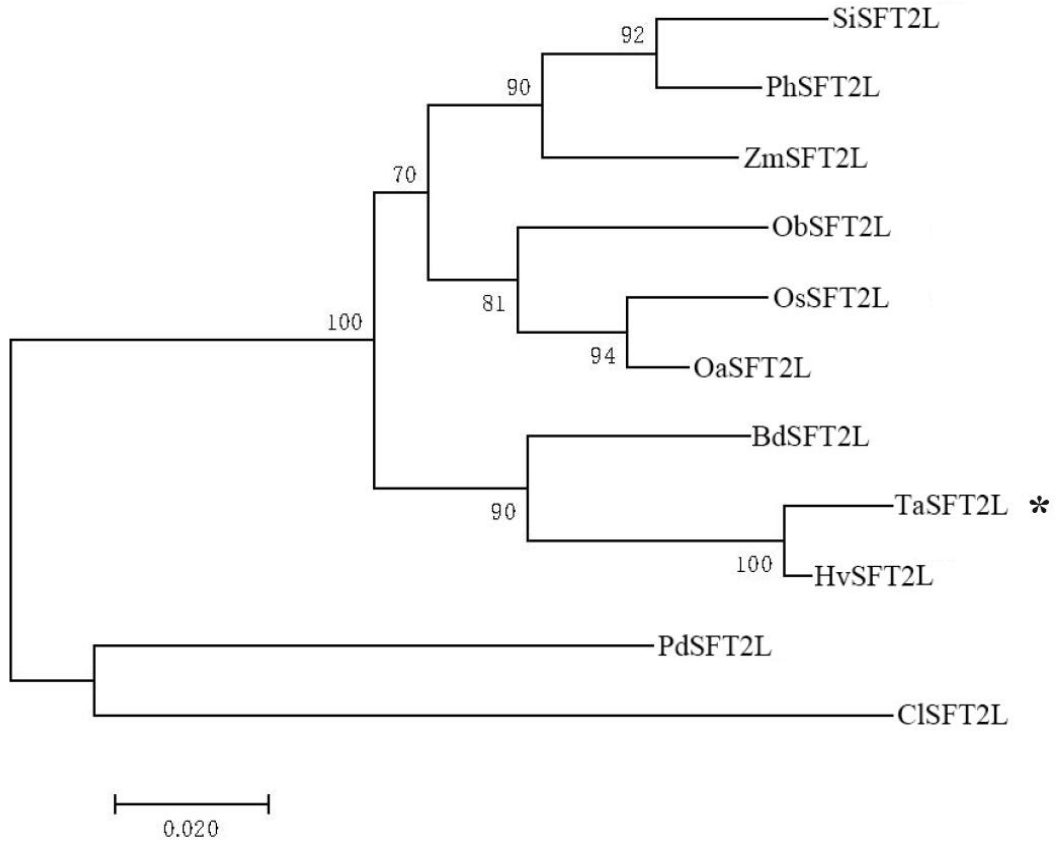


Figure 4.7. Phylogenetic tree of TaSFT2L using MEGA 7.0. The accession numbers are as follows: SiSFT2L (XP_004965916.1), PhSFT2L (XP_025812579.1), ZmSFT2L (ACG39380.1), ObSFT2L (XP_006656231.1), OsSFT2L (ACN85162.1), OaSFT2L (ACN85266.1), BdSFT2L (XP_014752367.1), TaSFT2L (XP_020198755.1), HvSFT2L (KAE8811045.1), PdSFT2L (XP_008782994.1), ClSFT2L (KAF3321481.1).

4.2.3. *TaSFT2L* expressions in wheat tissues

TaSFT2L was frequently expressed in wheat tissues, and was highly abundant in the roots (Figure 4.8A). Exposure to Cd over 24 h significantly induced *TaSFT2L* expressions in the root tip (Figure 4.8 B.).

To establish subcellular localization of TaSFT2L, P35S - TaSFT2L - GFP and TaPDA62 - 1301 - RFP fusion expression vectors were constructed using the 35S promoter, after which they were transferred into wheat mesophyll protoplasts (Figure 4.8 C). TaPDA62 - 1301 - RFP was the cytomembrane marker. Confocal microscopy revealed that TaSFT2L - GFP was localized on the cell membrane (Figure 4.8D).

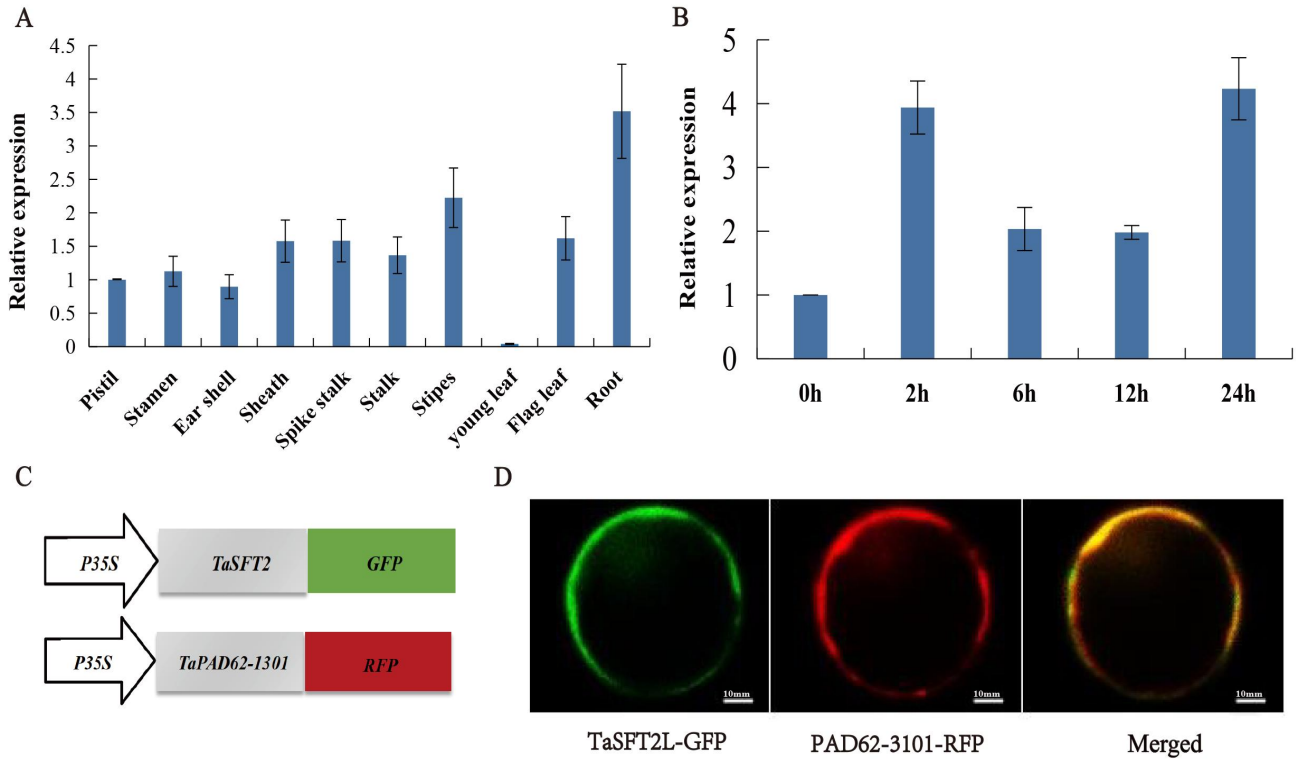


Figure 4.8. Expression pattern and subCellular localization of TaSFT2L in wheat. (A) The expression level of TaSFT2L in different tissues; (B) The data were normalized with TaSFT2L expression in root with 0.5 mM Cd stress for 24 h; (C) Schematic diagrams of fusion constructs; (D) Green fluorescence of TaSFT2L - GFP fusion protein; Red fluorescence of TaPAD62 - 1301 - RFP; Merged photo. Scale bar = 10 mm.

4.3. Complementation test of the yeast mutant of *TaSFT2L*

4.3.1. Complementation test of the yeast mutant of *TaSFT2L*

To understand whether *TaSFT2L* can transport or detoxify metals, we leveraged yeast mutant cell models for studying fundamental processes of metal uptake and detoxification. Cell functions of *TaSFT2L* were established by the primary yeast complementation assay. The pYES2 and empty *TaSFT2L* vectors were heterologously transferred into *ycf1* (*Saccharomyces cerevisiae* mutant strains) for growth comparisons. Under normal conditions, cell growth differences between wild - type and *TaSFT2L* expressing cells were not significant. Upon supplementation of 10 and 15μm Cd to the growth medium, the *TaSFT2L* growth phenotype was worse than WT and *ycf1* (Figure 4.9.).

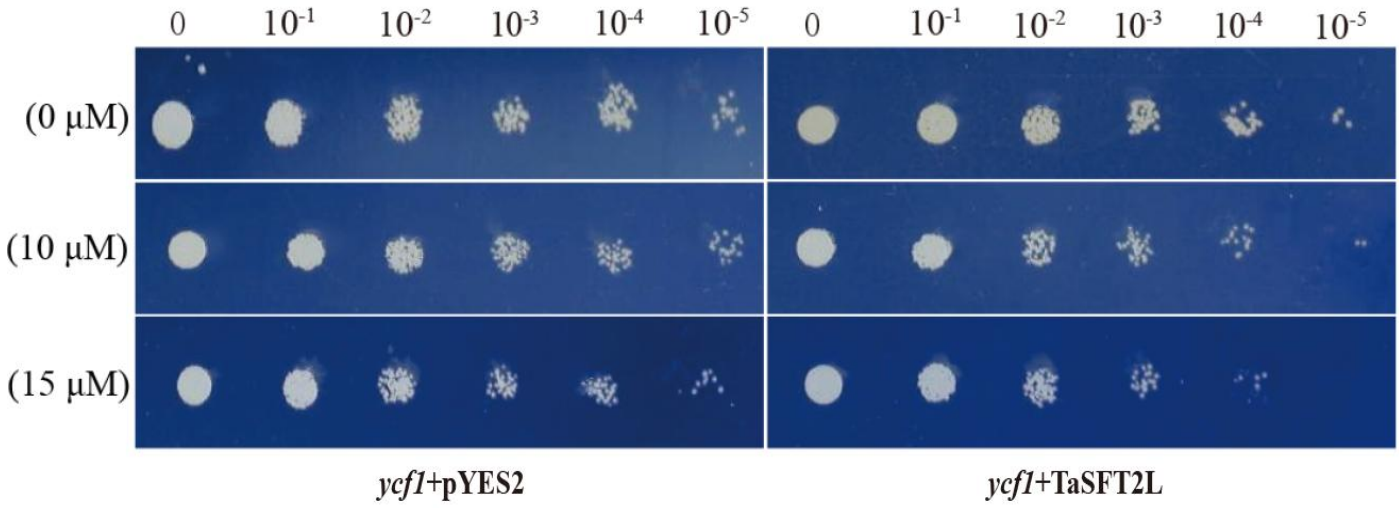


Figure 4.9. The effect of *TaSFT2L* expression on the tolerance of Cd in yeast. The mutant strain ycf1 were transformed with the empty vector pYES2 or *TaSFT2L*. Serial dilutions (1:10) of the yeast cells were spotted on the SG - Ura medium which contains different Cd (0, 10, 15μm) concentrations.

4.3.2. The growth rate of TaSFT2L was reduced under cadmium stress in in yeast strains

To confirm this finding, we evaluated the growth rates of the equivalent yeast strains under Cd stress in broth (Figure 4.10A). Cell density in ycf1 - *TaSFT2L* was lower, relative to that transformed with the empty pYES2 vector and supplemented with 10µm Cd. Notably, compared to WT, cells transformed with ycf1 - *TaSFT2L* displayed worse growth and tolerance to Cd. ycf1 - *TaSFT2L* under Cd stress was further examined. Cd contents were analyzed, the ycf1 - *TaSFT2L* expressing cells were 2.1 folds higher than the control of ycf1 (Figure 4.10B). These findings imply that ycf1 - *TaSFT2L* expression is associated with worse Cd tolerance in yeast cells.

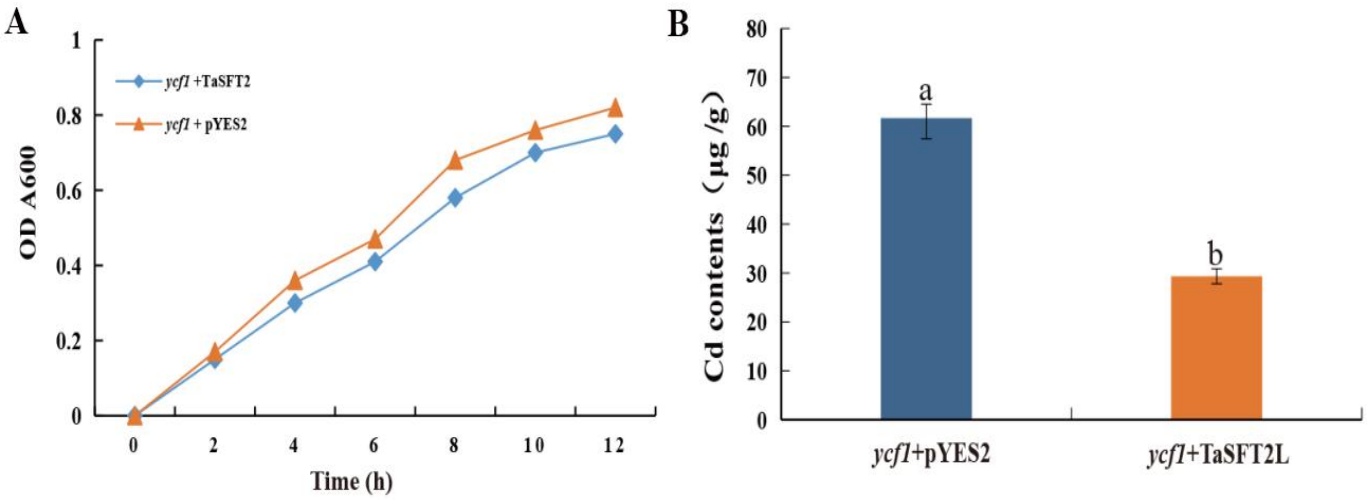


Figure 4.10. Growth of *TaSFT2L* in yeast. (A) Growth rate of yeast cells. The mutant strain ycf1 transformed with the empty vector pYES2 or *TaSFT2L* and wild - type were grown in SG - Ura medium supplemented with 10µm Cd and their cell densities determined at a specified period; (B) Cd concentrations in ycf1 mutants transformed with empty vector pYES2 or *TaSFT2L* grown under control or excess (10µm) Cd for 2 d. Different letters (a - f) indicate significant differences ($p < 0.05$).

After transformation of *TaSFT2L* into mutant *SFT2* yeast cells, autophagosomes in vacuoles were assessed to establish autophagic activities. At 5 h - post 1mM PMSF treatment and nutrient starvation, the wild - type yeast cells exhibited a high abundance of vacuolar autophagosomes, which were more accumulated in mutant *SFT2* yeast cells (Figure 4.11). In mutant *SFT2* yeast cells transformed with *TaSFT2L* rarely observed the autophagosomes (Figure 4.11).

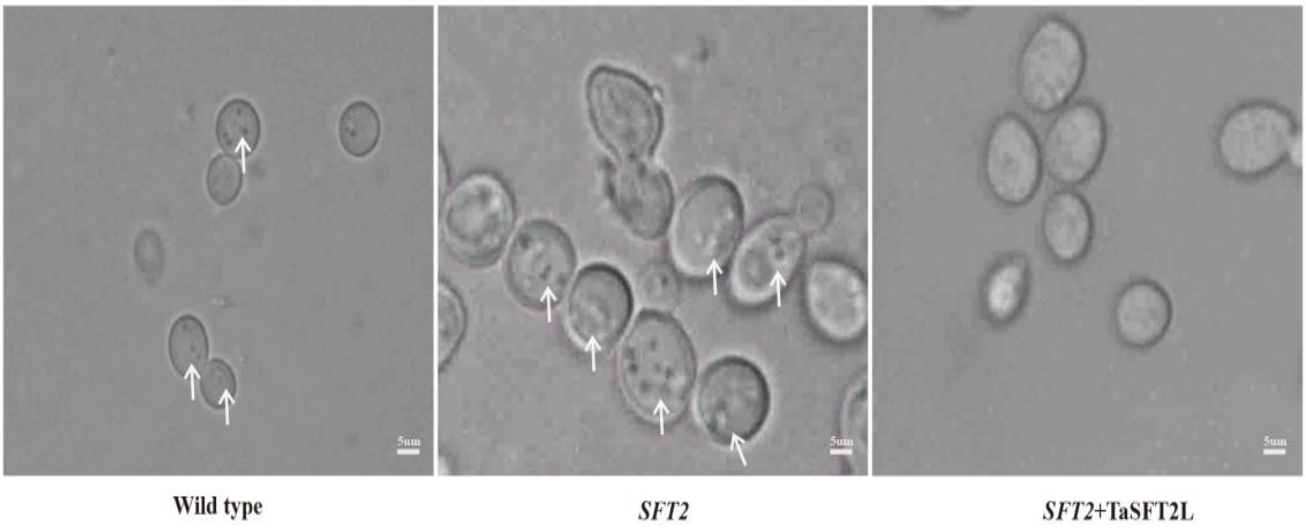


Figure 4.11. Functional complementation of *SFT2* - mutant yeast cells by wheat *TaSFT2L*.The autophagosomes accumulated within vacuoles are indicated by arrows, Scale bars represent 5µm. (Statistical comparison was performed by Duncan's test ($P < 0.05$).

4.4. TaSFT2L prokaryotic expression and growth analysis under Cd stress

TaSFT2L fusion protein expression in BL21 has been identified (DE3). *E. coli Rosetta* (DE3) was used to express recombinant pET - 28b - TaSFT2L after being 1mM IPTG-induced for 4 hours. SDS-PAGE was used to find the recombinant protein, as illustrated in Figure 4.12. The recombinant protein's theoretical

molecular weight, which was close to 24 KD and matched the electrophoresis result, showed that the IPTG-induced protein was successful.

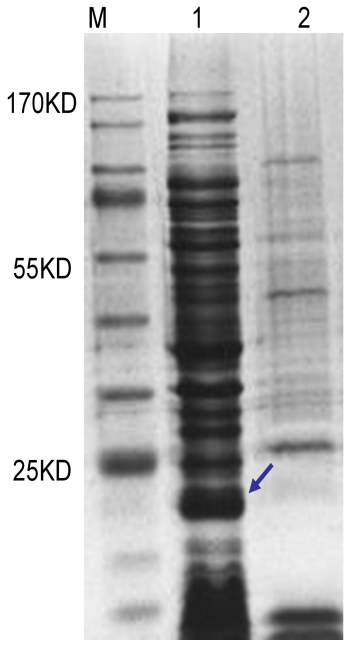


Figure 4.12. 30% (W/V) SDS - PAGE analysis of TaSFT2L expression and purification in *E. coli Rosetta* (DE3) cells. M: protein marker; 1: Induction with 1mM IPTG for 4h; 2: No IPTG induction.

4.5. The TaSFT2L fusion protein under cadmium stress

In order to confirm the role of TaSFT2L prokaryotic expression to Cd stress, we observed the growth of *E. coli Rosetta (DE3)* transformed by empty vector pET-28b, pET-28b - TaSFT2L, and IPTG-induced pET-28b - TaSFT2L (pET-28b - TaSFT2L-IPTG) under Cd stress. Figure 4-13 shows that under both low concentration Cd stress and normal conditions, the three cells (pET-28b, pET-28b-TaSFT2L, and pET-28b-TaSFT2L-IPTG) demonstrated comparable growth trajectories (Figure 4.13A and Figure 4.13B). The growth curves of the pET-28b, pET-28b - TaSFT2L, and pET-28b - TaSFT2L - IPTG transformed cells

under the three Cd concentrations (0 mM, 0.25 mM, and 0.5 mM) revealed substantial variations with the extension of concentrations and time. pET - 28b - TaSFT2L - IPTG protein cells developed more slowly than cells without TaSFT2L expression as the concentration of Cd increased (Figure 4.13C). The ability of cells under Cd stress to develop was reduced when they expressed TaSFT2L protein, which was produced by IPTG. This shows that pET - 28b - TaSFT2L may be crucial for Cd stress adaption.

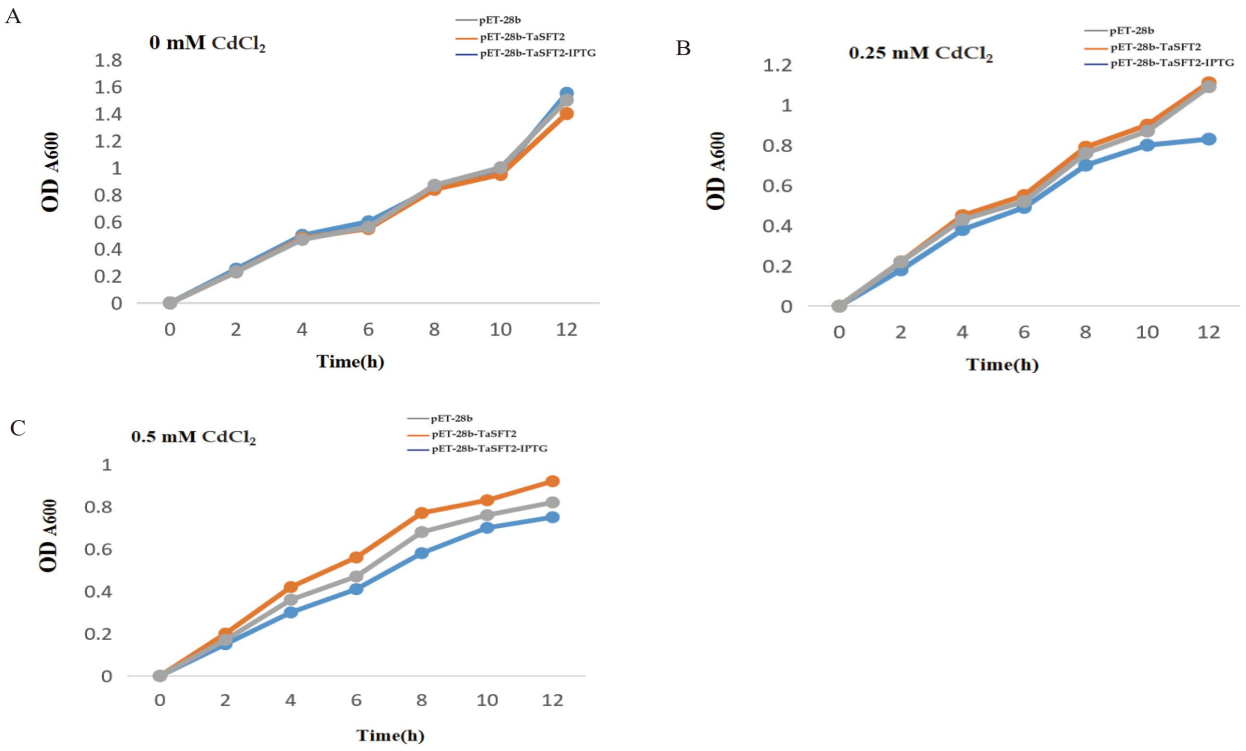


Figure 4.13. Comparison of the growth of pET - 28b - TaSFT2L transformed E. coli Rosetta (DE3) and empty vector (pET - 28b) without metal damage or different concentrations of cadmium stress. (A - C) The growth curves of *Escherichia coli* cells transformed by pET - 28b, pET - 28b - TaSFT2L and pET - 28b - TaSFT2L - IPTG were gray, red and blue, respectively. pET - 28b is empty vector; pET - 28b - TaSFT2L group refers to the vector of TaSFT2L transformation; pET - 28b - TaSFT2L - IPTG group refers to vectors transformed by TaSFT2L and induced by IPTG.

4.6. The growth of TaSFT2L fusion protein under different concentrations of cadmium

The tolerance of the recombinant TaSFT2L protein to Cd was demonstrated by observing colony growth in cultures containing 0mM, 0.25mM, and 0.5mM $CdCl_2$ (Figure 4.14). There was no significant difference in the growth of pET-28b, pET-28b - TaSFT2L, and pET-28b - TaSFT2L - IPTG without $CdCl_2$. The growth of pET - 28b no - load cells and IPTG induced protein cells (pET - 28b - TaSFT2L - IPTG) was significantly inhibited as Cd concentrations increased, particularly to 0.5mM $CdCl_2$. Cells lacking the IPTG-induced TaSFT2L protein (pET - 28b - TaSFT2L) were able to withstand high Cd concentrations.

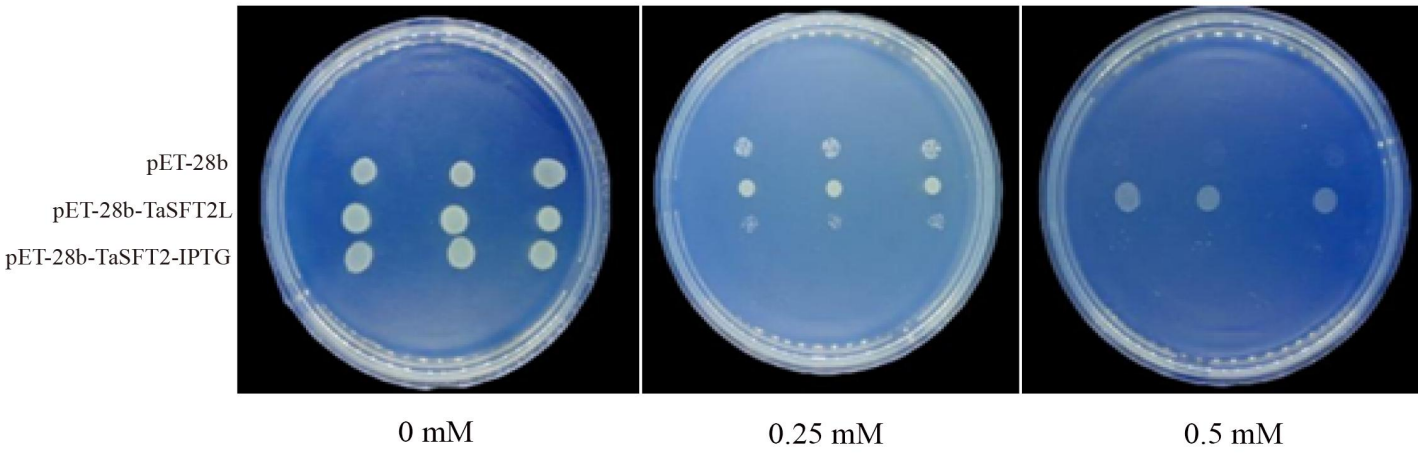


Figure 4.14. Spot detection of pET - 28b, pET - 28b - TaSFT2L, and pET - 28b - TaSFT2L - IPTG at 0 mM, 0.25 mM, and 0.5 mM $CdCl_2$ treatments.

4.7. Transgenic Arabidopsis expressing *TaSFT2L*

4.7.1. Establish *Atgn9* mutant

To establish the role of *TaSFT2L* in Cd stress responses, we acquired one T-DNA

insertion mutant of *TaSFT2L* as *Atgn9* (SALK_132905), which had T-DNA inserted into the 5′ untranslated region. Mutant genotypes were confirmed by PCR, and the complementary mutant material of *TaSFT2L* (*Atgn9* - OE) was obtained (Figure 4.15A). The qPCR analysis suggested that *Atgn9* was a knockout mutant.

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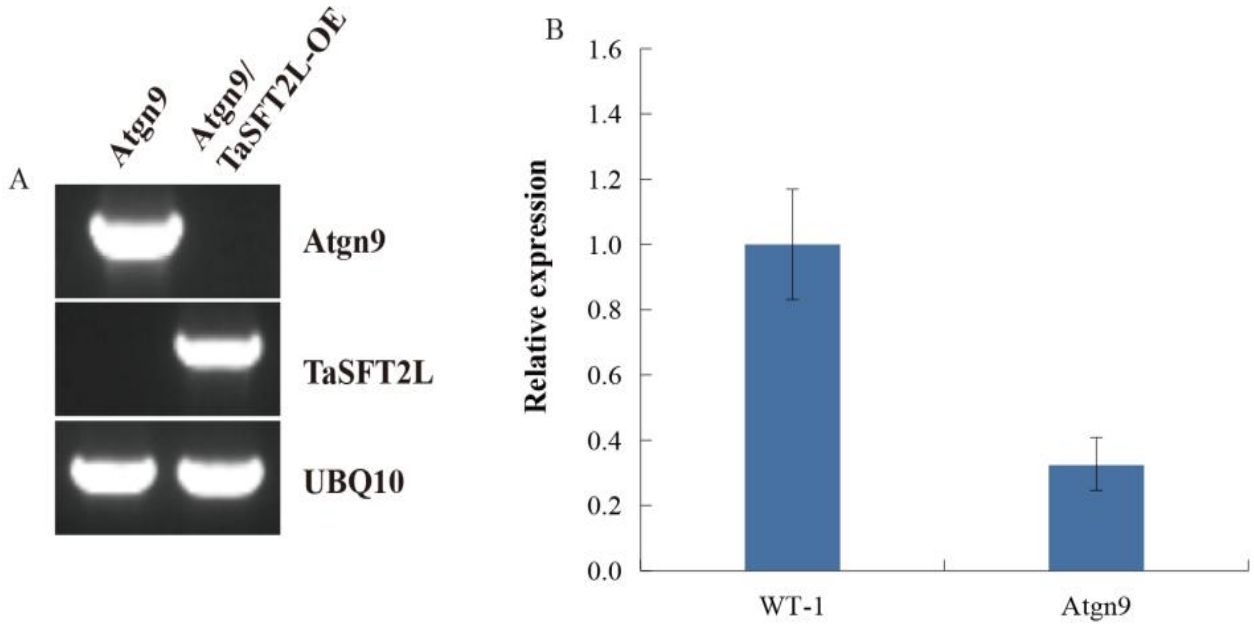

Figure 4.15. (A) Genotypic analysis of the *Atghn9* (SALK_132905) mutant alleles and complementary material by genotyping PCR. (B) The expression level of *Atgn9* in WT (Col) and the mutant. Different letters (a - f) indicate significant differences ($p < 0.05$).

4.7.2. Reduced Cd tolerance of transgenic *Arabidopsis* expressing *TaSFT2L*

In the Cd stress response assay, the *Arabidopsis thaliana* seeds were moved to MS plates containing 0 umol/L,50 umol/L, and 75umol/L $CdCl_2$. After 10 days of growth (Figure 4.16A) without $CdCl_2$, differences among *Atgn9* mutants, WT as well as complementary plants (*Atgn9* - OE) with regards to fresh weight and root length were not significant (Figures 4.16B and 4.16C), Interestingly, under Cd stress condtions, WT and complementary plants exhibited greater inhibition of growth with regards to fresh weight (Figure 4.16B) and root length (Figure 4.16C) than the *Atgn9* mutants. These findings show that *TaSFT2L* plays a vital role in seedling growth as a potential negative factor in tolerance to Cd stress.

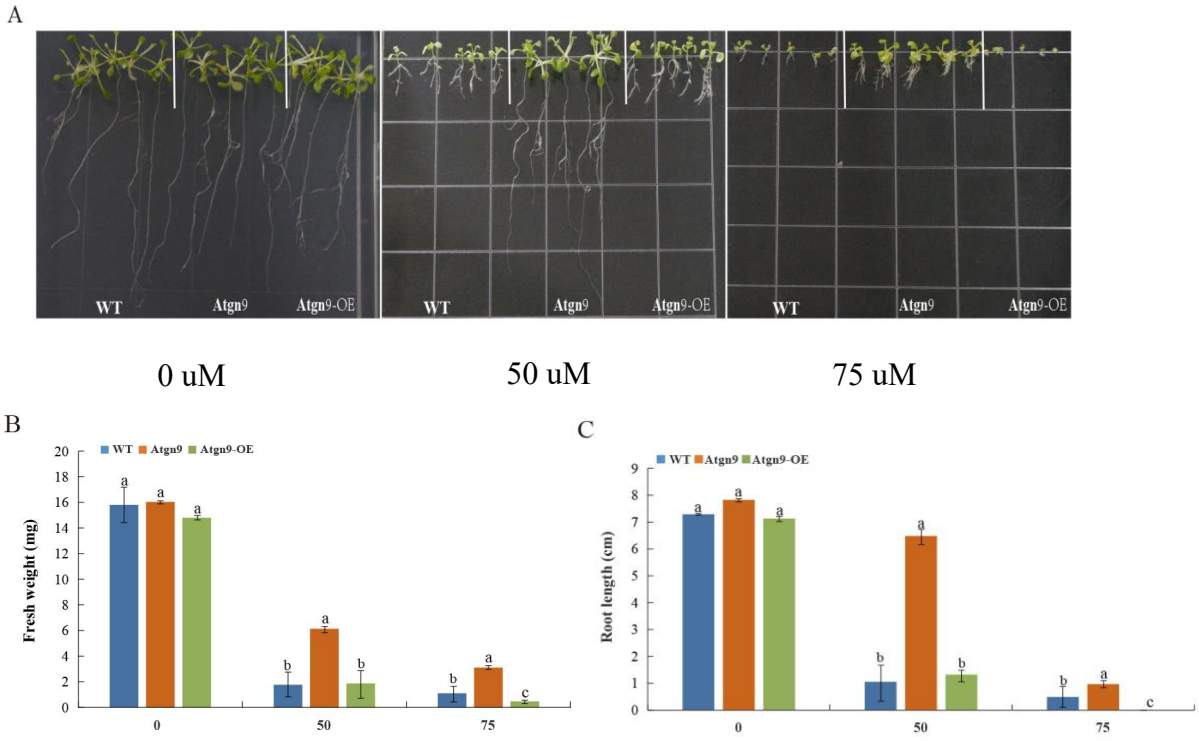

Figure 4.16. Cd tolerance of transgenic *Arabidopsis* expressing *TaSFT2L*. (A)The phenotype of Arabidopsis seedlings grown vertically on MS medium supplemented with 0, 50 and 75 umol/L $CdCl_2$ for 10 d; (B - C) Root length and fresh weight analysis of *Arabidopsis* seedlings. Different letters (a - f) indicate significant differences ($p < 0.05$).

4.8. Functional analysis of *TaSFT2L* in wheat

4.8.1. BSMV - VIGS inoculation and *TaSFT2L* function analysis

(1) BSMV system verification

The BSMV: *TaPDS* - inoculated plants exhibited significant photo - bleaching (Figure 4.17A). *TaPDS* transcription levels in BSMV: *TaPDS* - inoculated plants were inhibited by over 80%, relative to mock - inoculated plants (Figure 4.17B), implying that the BSMV - VIGS system is appropriate for gene silencing studies of *TaSFT2L* (Appendix D1).

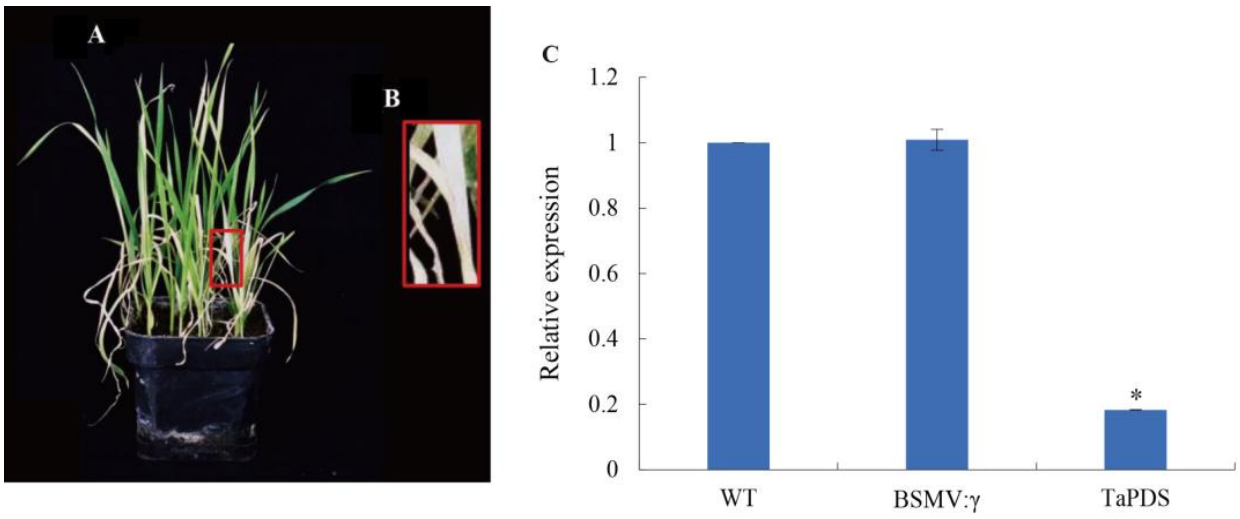

Figure 4.17. (A, B) Photo - bleaching phenotypes of BSMV: *TaPDS* - inoculated plants was taken at 14 days after vitro transcription. B is the enlarged visual field of A; (C) The expression levels of BSMV - mediated VIGS of *TaPDS* gene in wheat. Values are the mean±SE (n = 3); Asterisks indicate significant differences ($p < 0.05$).

(2) BMSV: *TaSFT2L* is beneficial to root growth

The expression levels of *TaSFT2L* in root tip of BSMV: *TaSFT2L* - inoculated

plants were 70% and 75% lower than WT and BSMV: γ - inoculated plants, respectively (Figure 4.18B). Interestingly, in the presence of 0.5 mM Cd, BSMV: *TaSFT2L* - inoculated plants exhibited significantly increased root growth compared with the wild type (Figure 4.18A). Root length and dry weight of the BSMV: *TaSFT2L* - inoculated seedlings showed no difference compared with those of the BSMV: γ - inoculated plants under control conditions (without Cd) (Figure 4.18C, D). However, the addition of 0.5 mM Cd in growth medium significantly increased root dry weight and root length by 47.61% and 34.97% in the BSMV: *TaSFT2L* - inoculated seedlings compared with the BSMV: γ - inoculated plants, respectively (Figure 4.18C, D).

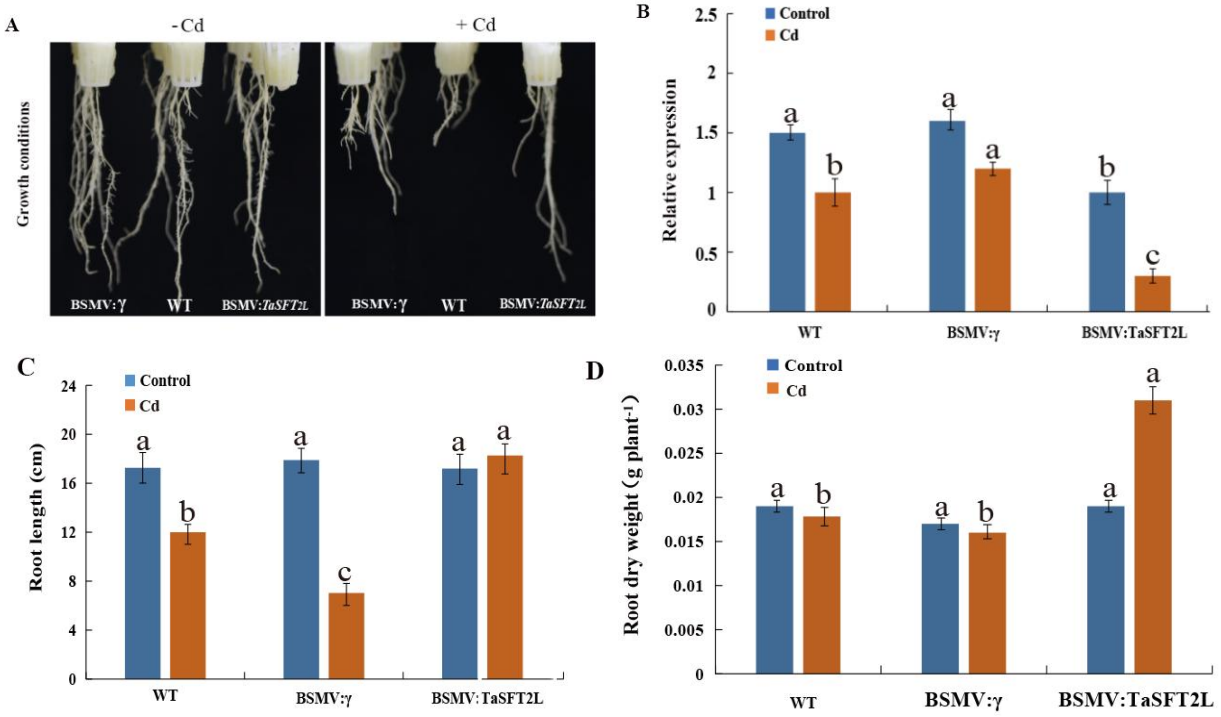

Figure 4.18. Phenotypic analysiss of BMSV: *TaSFT2L*. BSMV: γ and BSMV: *TaSFT2L* subjected to 0.5 mM Cd for 30 days. (A) Phenotypes of WT and BSMV lines with normal and 0.5 mM Cd condition; (B) The relative expression level of WT, BSMV: γ and BSMV: *TaSFT2L* in root of wheat; (C) Root activity; (D) Root lengthγ and BSMV: *TaSFT2L* in root of wheat Different letters (a - f) indicate significant differences ($p < 0.05$).

(3) BMSV: *TaSFT2L* enhances root activity

In addition, to assess oxidative stress levels in root cells under a toxic Cd environment and amelioration of root structural damage caused by the BSMV: *TaSFT2L* - inoculated plants, we assessed visual reactive oxygen species (ROS) distribution, cell viabilities and surface morphology as well as ultrastructures of roots. Under toxic Cd conditions, root activities were markedly enhanced, and red fluorescence in roots was markedly suppressed.

Clear green patches were observed in the roots of BSMV: *TaSFT2L* - inoculated plants (Figure 4.19A, B). The H_2O_2 and $O\cdot^-$ in the roots were fluorescence probe - stained to assess ROS accumulation - mediated oxidative damage to cytomembranes, compared with WT and BSMV: *TaSFT2L* - inoculated plants. As shown in Figure 4.19A, BSMV: *TaSFT2L* - inoculated plants had decreased fluorescence intensities and H_2O_2 levels in toxic Cd roots. These findings indicate that BSMV: *TaSFT2L* - inoculated plants contributed to amelioration of Cd - mediated oxidative stress, stress injury repair in cells, and high cell viabilities, to enhance root growths under a toxic Cd environment.

In Figure 4.20, Cd toxicity dysregulated wheat root morphologies, which resulted in significantly broken cell epidermis. However, the toxic Cd wheat roots' surface was normalized with the BSMV: *TaSFT2L* - inoculated plants, indicating that the silencing of *TaSFT2L* was important for maintenance of the integrity of surface structures of roots and for reduction of Cd toxicity.

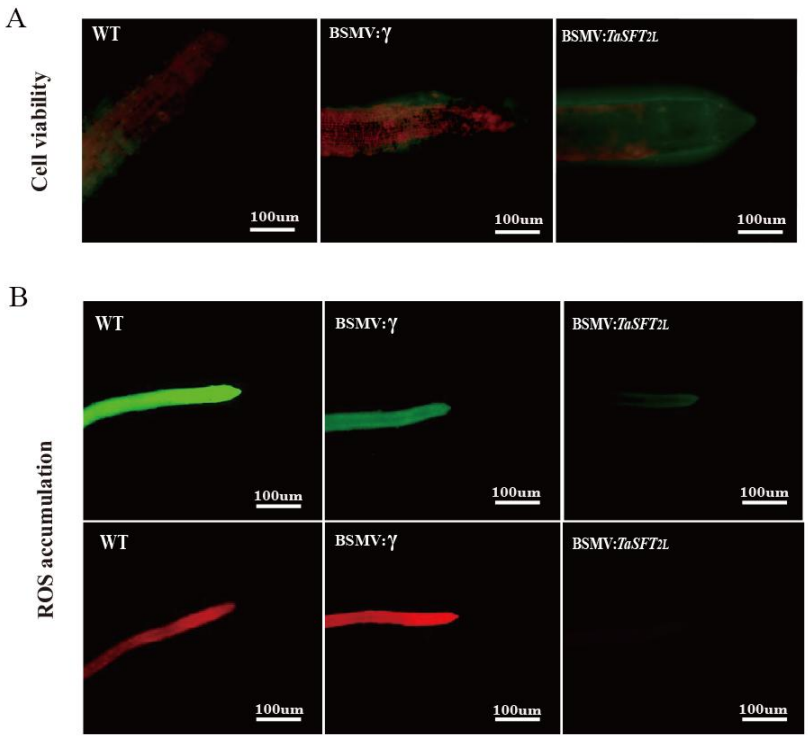

Figure 4.19. Cell viability and ROS accumulation, BSMV: γ and BSMV: *TaSFT2L* subjected to 0.5 mM Cd for 30 days. (A) cell viability, Bars 100 μm; (B) ROS accumulation, Bars 100μm.

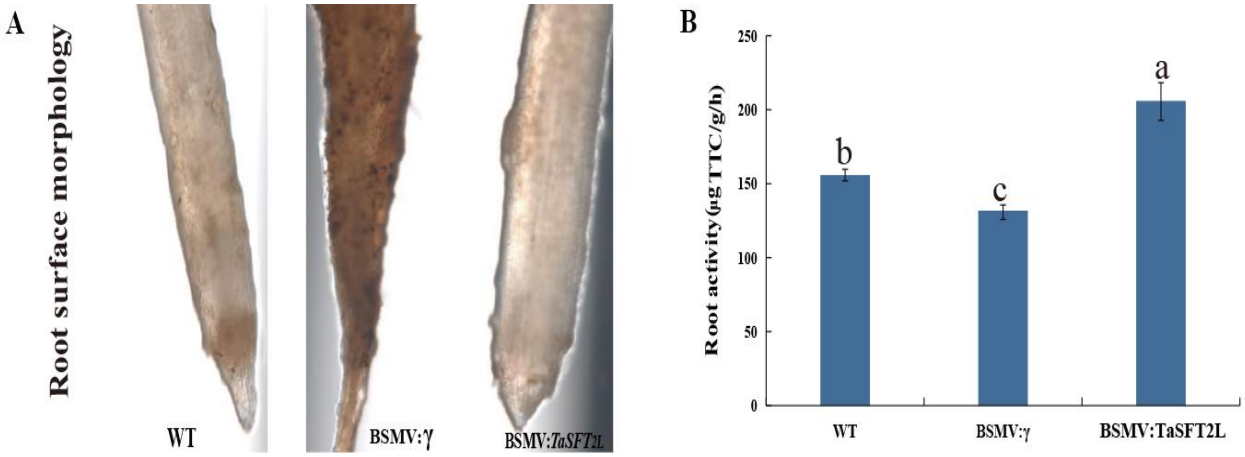

Figure 4.20. Root morphology. BSMV: γ and BSMV: *TaSFT2L* subjected to 0.5 mM Cd for 30 days. (A) Root morphologies; (B) Root activity; Different letters (a - f) indicate significant differences ($p < 0.05$).

(4) BMSV: *TaSFT2L* increases autophagosomes

We inoculated BSMV - treated plants with 0 and 0.5 mm Cd and determined their constitutive autophagy activity via LysoTracker Red staining. This dye stains acidic organelles, including endosomes, lysosomes as well as autophagosomes. For the BSMV - inoculated plants with 0.5 mm Cd, a LysoTracker Red - stain punctate pattern was detected in Cd - treated root epidermal cells (Figure 4.21). These Cd - enhanced levels imply that silenced *TaSFT2L* - regulatory autophagic processes were induced. However, without Cd stress, the stained punctate structures were not detected in BSMV leaf samples: γ - treated plants (Figure 4.21). In addition, root cells were observed by transmission electron microscope (TEM). We observed strong autophagic activities in BSMV roots: *TaSFT2L* - inoculated plants, which was substantiated by the fact that the *TaSFT2L* formed more frequently under Cd treatment (Figure 4.22). In plants, the role of autophagy in degradation and recycling of damaged proteins as well as organelles is vital in abiotic stress environments [231].

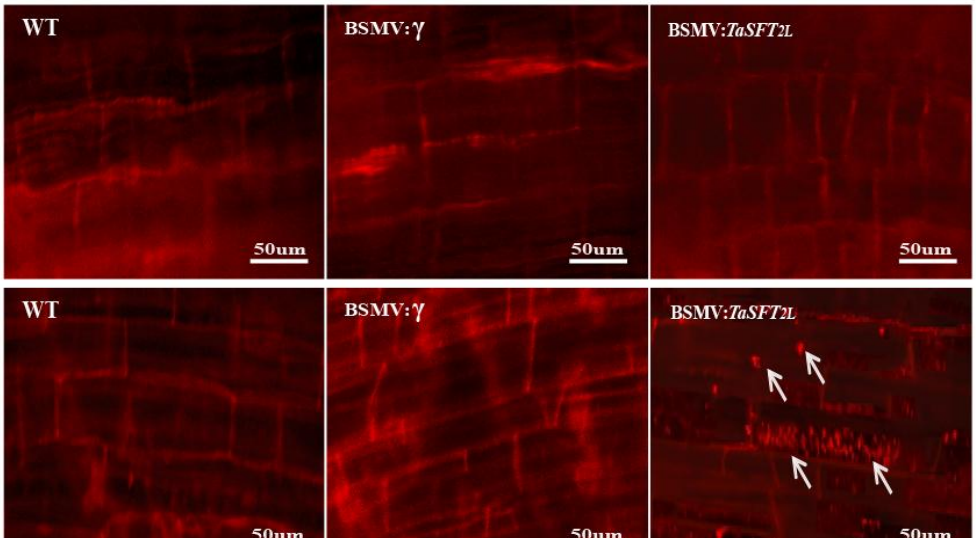

Figure 4.21. Autophagy is induced in the BSMV: *TaSFT2L* - inoculated plants response to Cd stress. LysoTracker Red - stained punctate autolysosomes structures are indicated by arrows, Bars 50 μm.

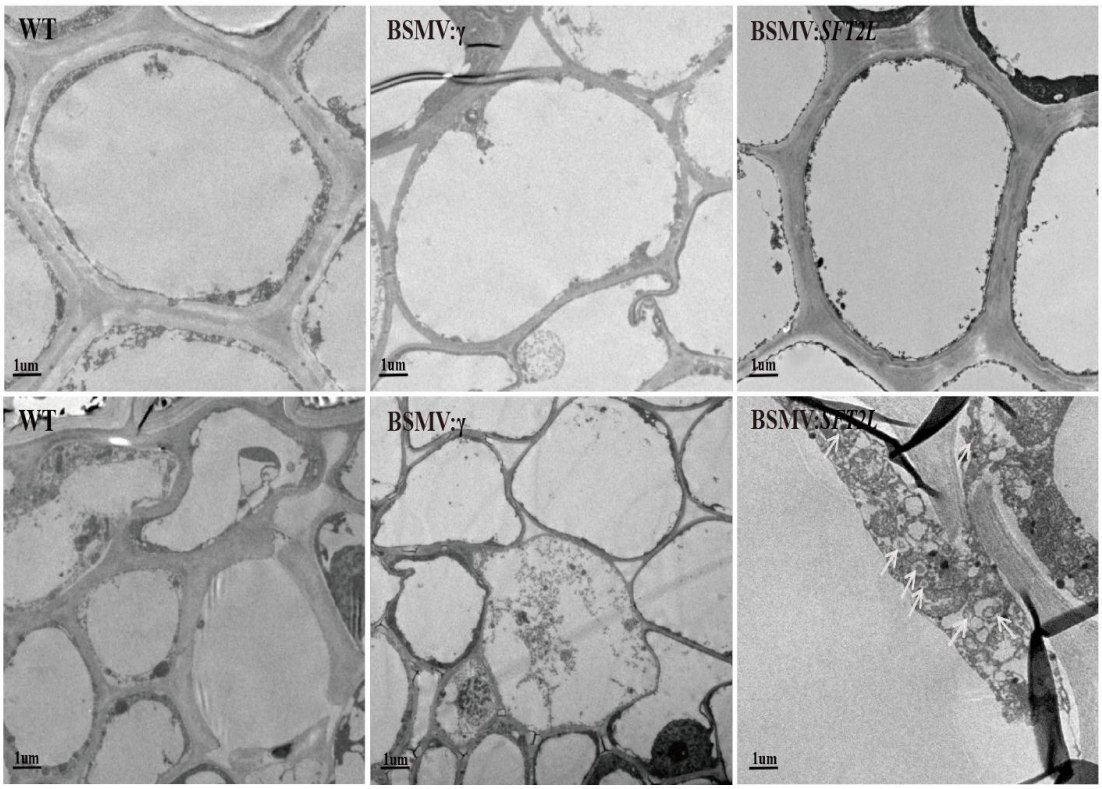

Figure 4.22. TEM were observed and representative images were chosen for analysis; The arrows indicate autophagosomes, Bars 1 μm.

(5) BMSV: *TaSFT2L* reduced cadmium content in wheat

This study demonstrated elevated autophagic activities in roots, which were attributed to silenced *TaSFT2L* in wheat could be associated with the enhanced tolerance to Cd toxicity. To establish if Cd absorption was affected in BSMV: *TaSFT2L* - inoculated plants, we investigated their roots contents. We evaluated Cd abundance in roots. It was revealed that BSMV: *TaSFT2L* - inoculated plants significantly decreased Cd accumulations, compared with WT and BSMV: *γ* - inoculated wheat seedlings (Figure 4.23A). We studied the regulation of BSMV: *TaSFT2L* - inoculated plants on subcellular localization of Cd in wheat roots and evaluated Cd levels in different cell portions. BSMV: *TaSFT2L* - inoculated plants treated for Cd toxicity remarkably exhibited increased Cd content in the soluble

fractions and organelles, however, cell wall Cd levels were not affected (Figure 4.23B, C, D). With the silencing of *TaSFT2L,* Cd subcellular distribution in wheat root soluble fractions and organelles respectively decreased by 22.50% and 38.20%, compared to BSMV: γ - treated plants.

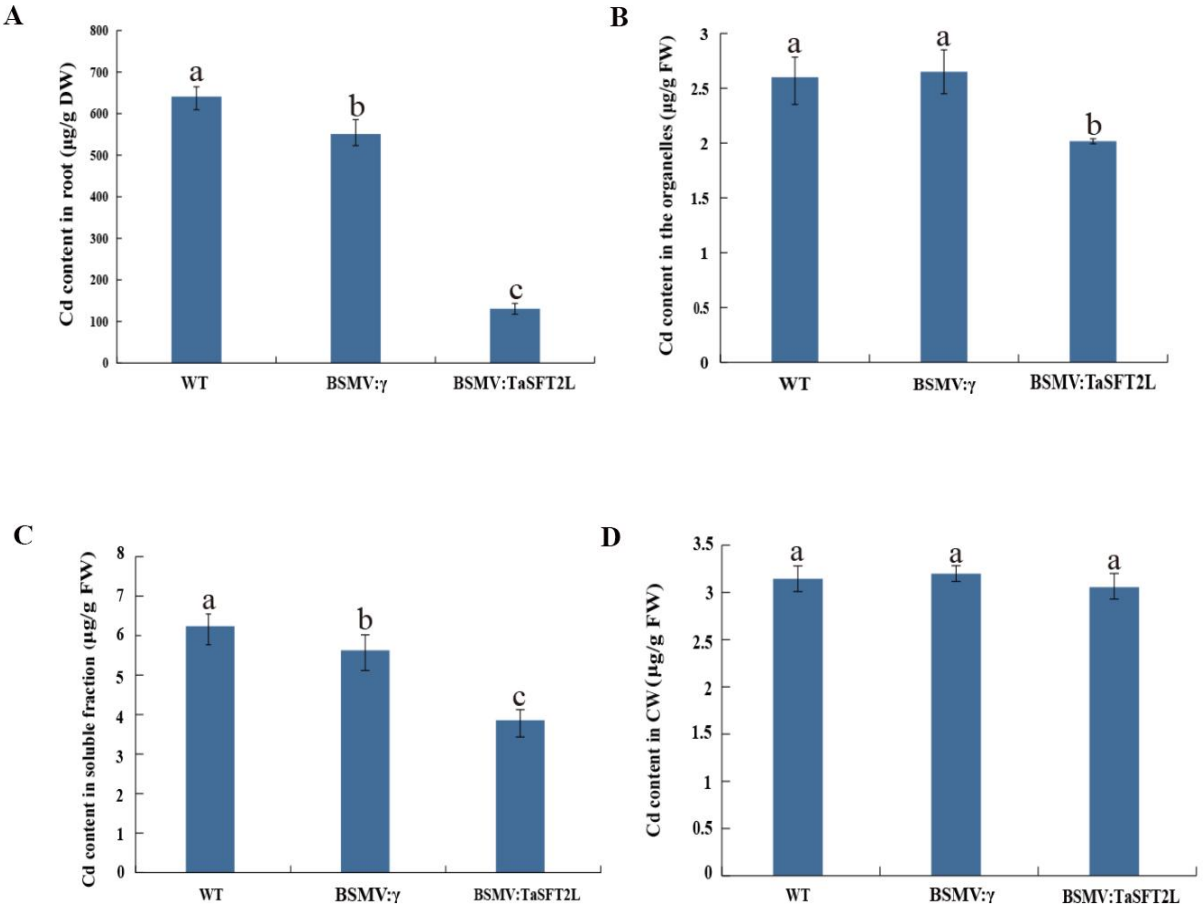

Figure 4.23. Cd content in roots, BSMV: γ and BSMV: *TaSFT2L* subjected to 0.5 mM Cd for 30 days. (A) Cd content in roots; (B - D) Content of Cd in different cell fractions; Different letters (a - f) indicate significant differences ($p < 0.05$).

4.8.2. Functional analysis of *TaSFT2L* gene in transgenic plants

(1) Identification of transgenic plants

PCR detection was used to obtain DNA from wheat positive strains. Meanwhile, DNA from WT and ddH_2O was extracted and amplified using PCR

technology as a negative control. Figures 4.24A, B show the electrophoresis results. There were no bands in WT or ddH_2O, but 450 bp and 750 bp bands were obtained by DNA amplification of RNAi, OE transgenic plants, and WT control plants, respectively, and were consistent with the expected size. Three RNAi transgenic plants and eight OE transgenic plants were obtained. The establishment of a wheat genetic transformation system ensures the functional analysis and regulation mechanism of wheat genes.

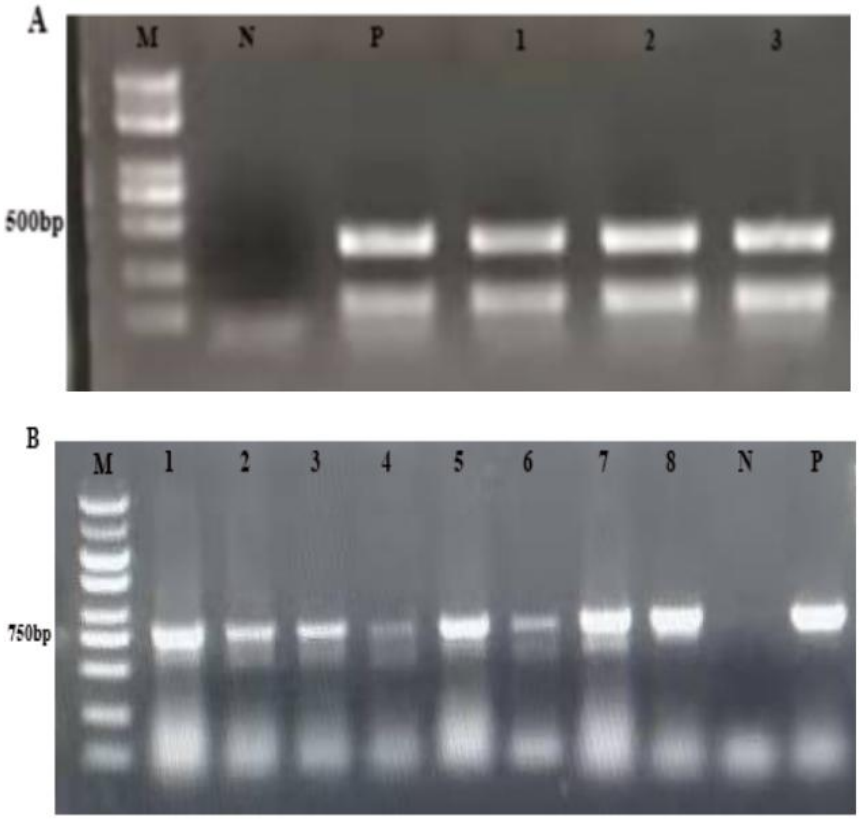

Figure 4.24. Positive transgenic wheats by PCR. (A) RNAi positive plant identification. (B) OE positive plant identification.M. Standard 2000+Marker; 1 - 8, positive plant identification; N, Negative control; P, positive control.

(2) Phenotypic analysis of RNA interference (RNAi) lines of *TaSFT2L* at different cadmium concentrations

To confirm the functional role of *TaSFT2L*, transgenic wheat RNAi was constructed (homozygous, T2). Four Cd doses (0, 0.5, 2, and 10 mm) were employed in treatment of transcripts of RNAi4, RNAi6 and RNAi7 and wild - type for seven days. Three randomly selected RNAi lines revealed that, under varying concentrations, expression levels of RNAi lines were 66.36 - 94.22% lower than those of the wild - type (Figure 4.25) (Appendix D2). Although growth responses of the RNAi lines and WT were similar under normal conditions (Figure 4.26A), the RNAi lines grew better than the WT under Cd stress. When exposed to 2 - 10 mm Cd, they had longer shoot and root growth than the WT (Figure 4.26B, C). Comparable findings were obtained for dry biomass (Figure 4.26D, E). The shoot and root biomass of the RNAi lines was 19.4 - 42.9% and 22.1 - 37.5% greater than that of the wild - type under 2 - 10 mm Cd exposure, respectively.

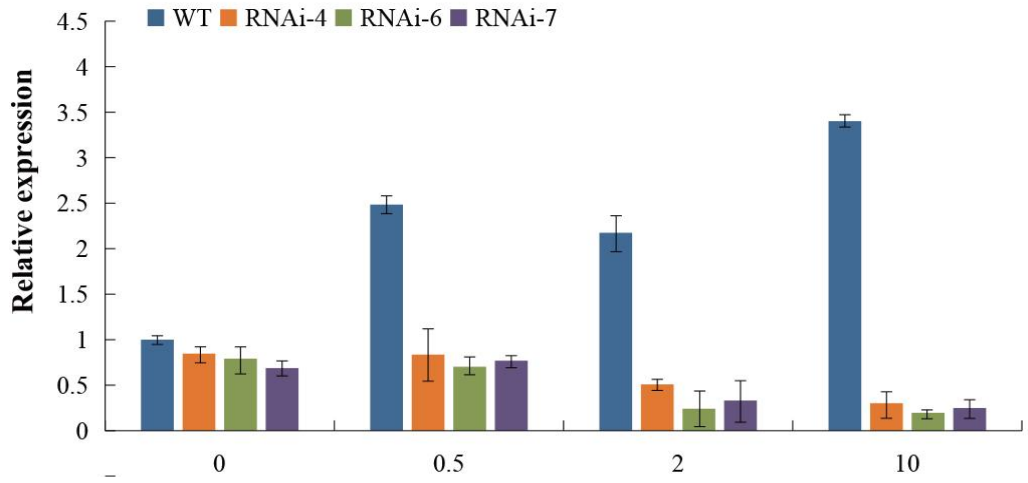

Figure 4.25. The expression level of *TaSFT2L* RNAi lines in different concentrations (0, 0.5, 2, 10mM) of Cd. Statistical comparison was performed by Duncan's test ($P < 0.05$).

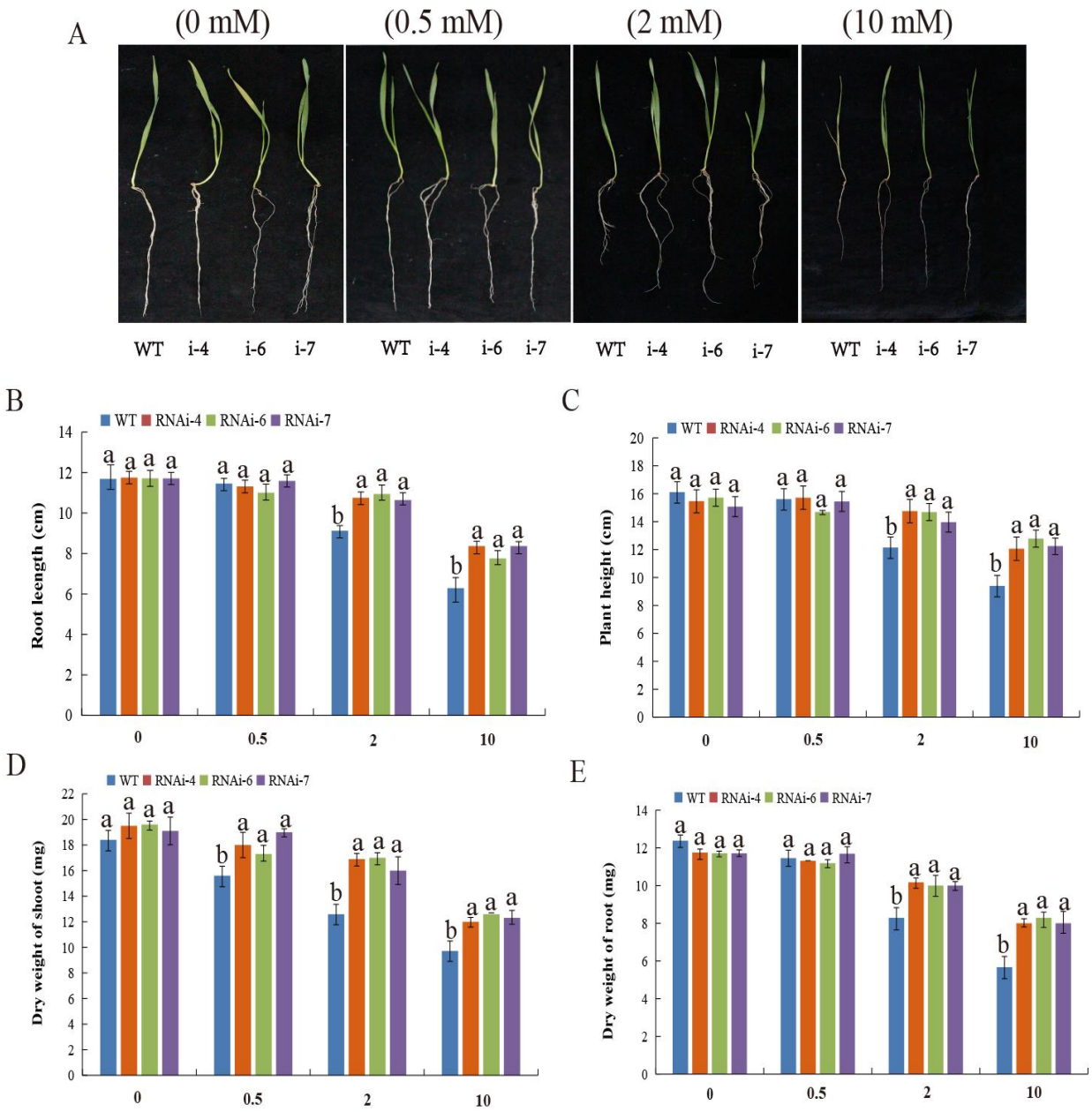

Figure 4.26. The phenotype of *TaSFT2L* RNAi lines and wild - type (WT) under Cd stress. The 10 d old *TaSFT2L* RNAi lines and WT were transferred to a nutrient solution containing different Cd concentrations (0, 0.5, 2, 10 mM Cd) for 7 d. (A) Phenotypes of RNAi lines and WT; (B) Root length; (C) Plant height; (D) Dry weight of shoot; (E) Dry weight of root. Different letters (a - f) indicate significant differences ($p < 0.05$).

(3) Phenotypic analysis of overexpression (OE) lines of *TaSFT2L* at different cadmium concentrations

Additionally, for functional studies, we randomly selected three overexpression (OE) lines of *TaSFT2L* as OE2, OE3 and OE4. Transcript evaluation revealed that levels of the 3 OE lines were markedly higher than WT by 1.27 - 2.85 folds under 2 - 10mm Cd (Figure 3.27) (Appendix D2). Overexpression lines of *TaSFT2L* were treated with same Cd doses for 7 d. Under normal conditions, growth differences between the three OE lines and WT were not significant (Figures 4.28A , B). However, it was observed that the OE lines were worse than WT under 2 - 10 mm Cd (Figure 4.28B, C). Meanwhile, the shoot as well as root lengths and dry biomass were markedly lower, relative to the wild type (Figure 4.28D, E).

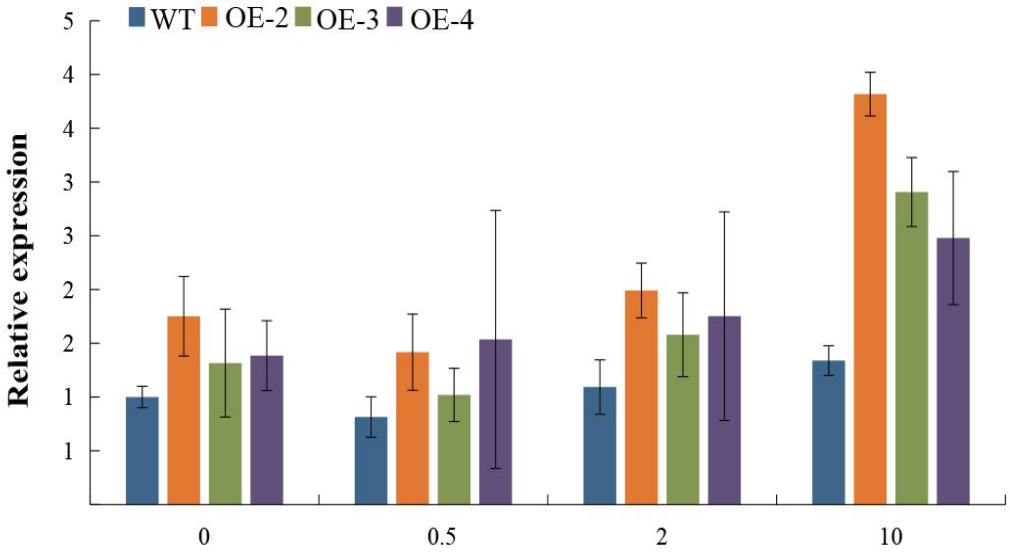

Figure 4.27. The expression level of *TaSFT2L* OE lines in diffffferent concentrations (0, 0.5, 2, 10mM) of Cd. Statistical comparison was performed by Duncan’ s test ($P < 0.05$).

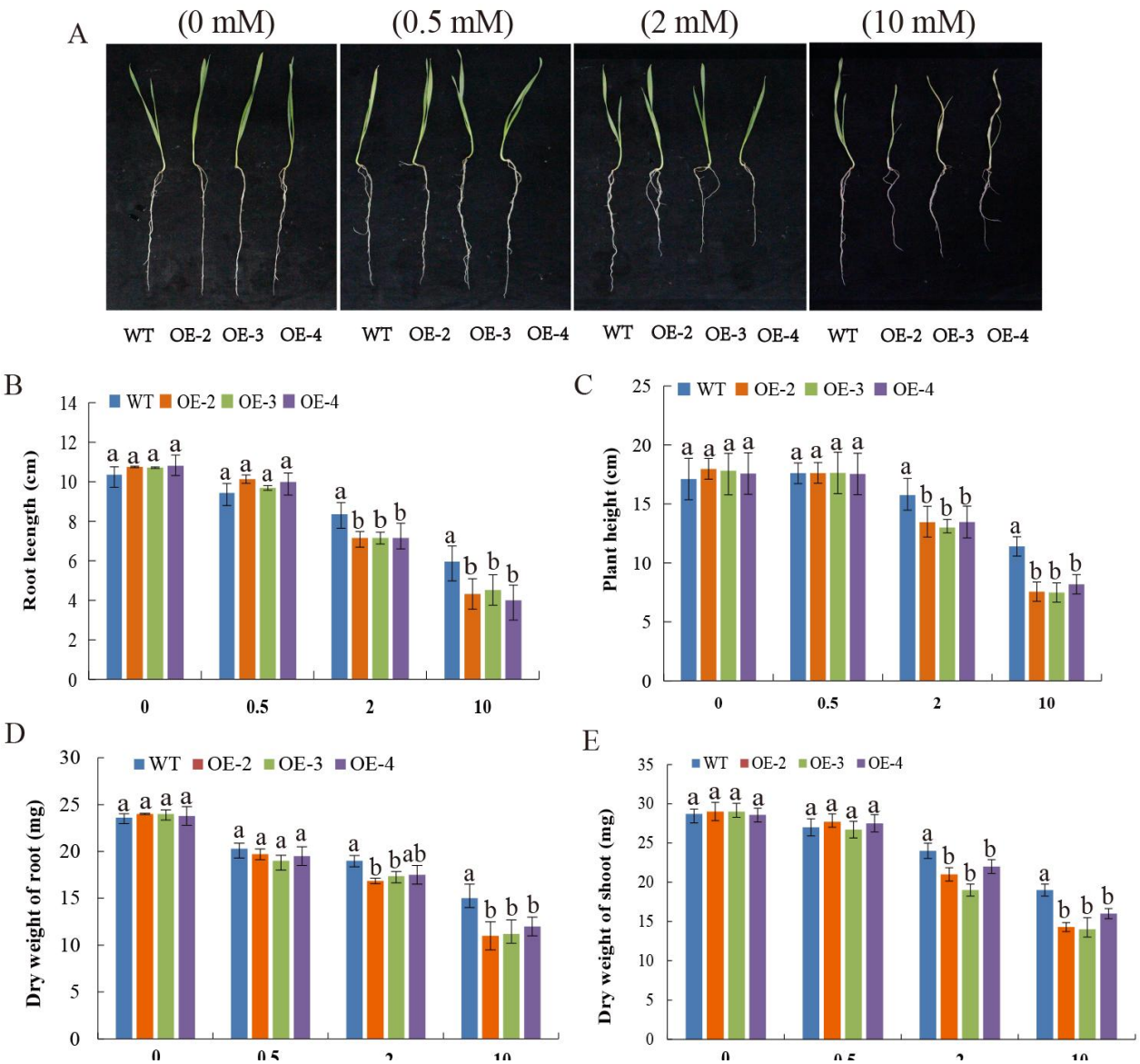

Figure 4.28. The phenotype of *TaSFT2L* OE lines and wild - type (WT) under Cd stress. The 10 d old *TaSFT2L* overexpression lines and WT were transferred to a nutrient solution containing different Cd concentrations (0, 0.5, 2, 10 mM Cd) for 7 d. (A) Phenotypes of overexpression lines and WT; (B) Root length; (C) Plant heigh; (D) Dry weight of root; (E) Dry weight of shoot. Different letters (a - f) indicate significant differences ($p < 0.05$).

(4) The chlorophyll content of RNAi lines was increased, while that of OE was opposite

Chlorophyll is sensitive to environmental stresses, especially to excessive heavy metals, and thus is often used as an indicator for plant stress response [16]. The chlorophyll level was unaffected in the RNAi lines compared to the WT at low Cd concentrations (0.5 mm) (Figure 4.29A, B); When the Cd concentration rose to 10 mm, the doses of Chl - a, Chl - b in RNAi lines were increased to 28.79 - 80.83% of the WT (Figure 4.29A, B), while a reduction of chlorophyll concentration in OE lines was observed under 0.5 - 10 mm Cd (Figure 4.29C, D).

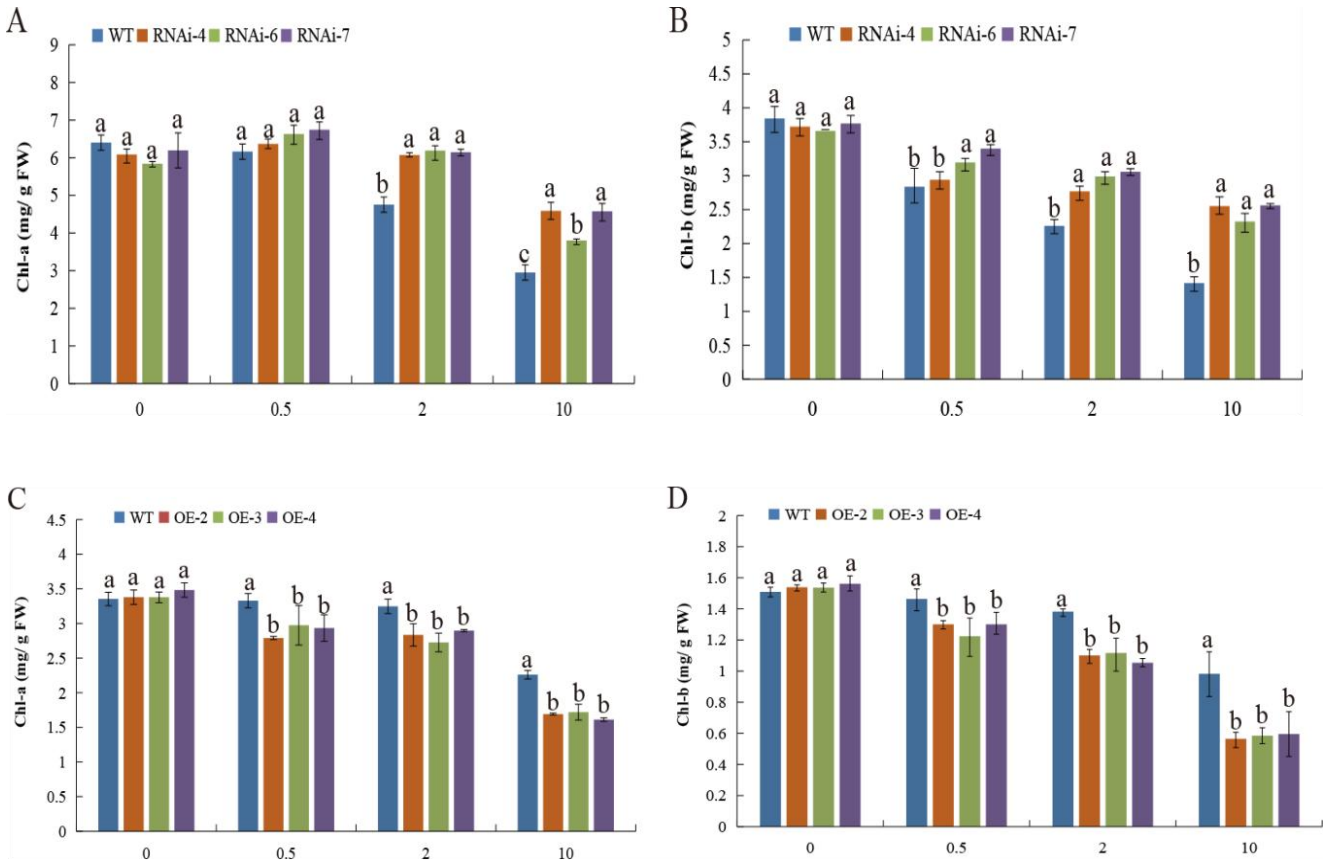

Figure 4.29. The Chlorophyll content of *TaSFT2L* transgenic lines and wild - type (WT) under Cd stress. The 10 d old *TaSFT2L* RNAi/ OE lines and WT were transferred to a nutrient solution containing different Cd concentrations (0, 0.5, 2, 10 mM Cd) for 7 d. (A - B) The Chlorophyll of RNAi lines; (C - D) The Chlorophyll of OE lines; Different letters (a - f) indicate significant differences ($p < 0.05$).

accumulation in wheat, the accuracy of the experiment was conducted by ICP - MS. Cd levels in RNAi lines were significantly lower than those of the WT under 2 - 10 mm Cd stress (Figure 4.30A and 4.30B). For instance, the Cd levels in the shoot and root were decreased by 16.34 - 48.09% at 10 mm of Cd. Conversely, the OE lines contained high Cd levels than the WT (Figure 4.30C and 4.30D).

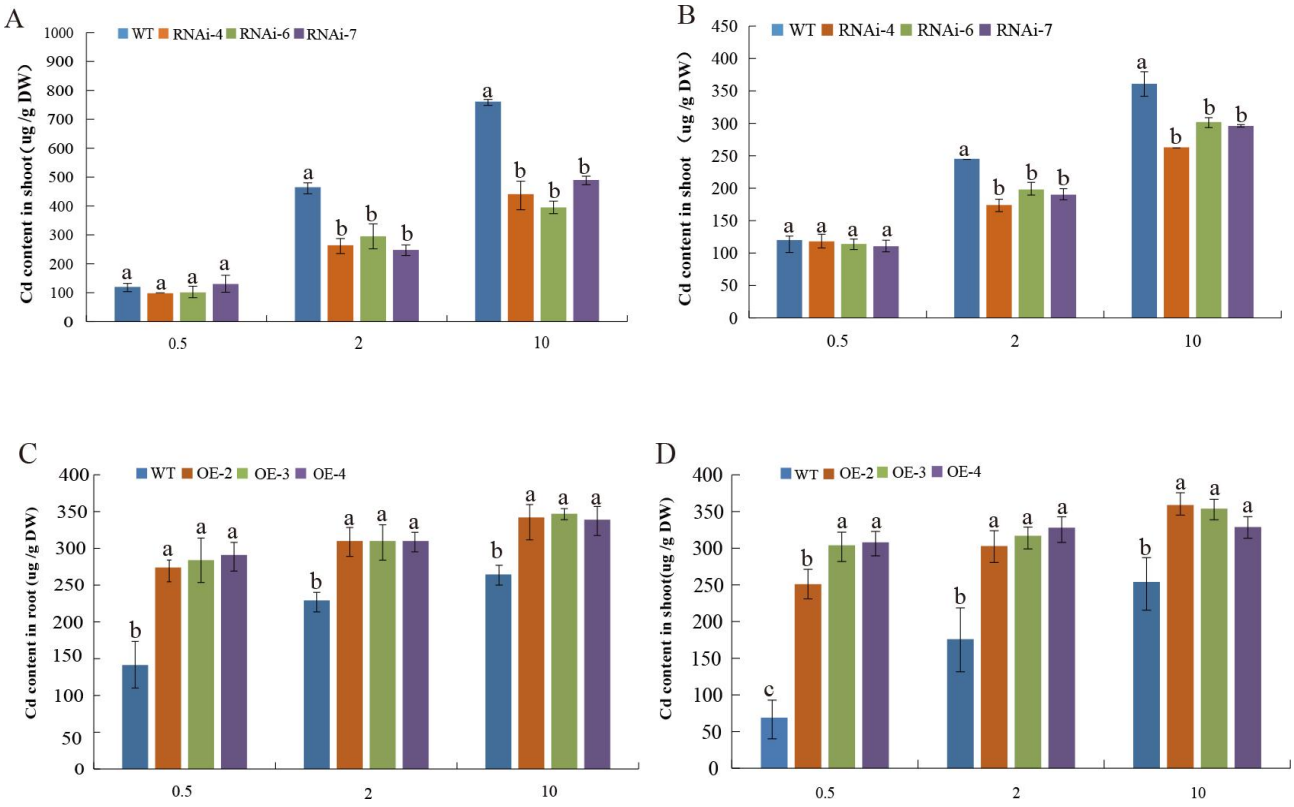

Figure 4.30. The Cd content of *TaSFT2L* transgenic lines and wild - type (WT) under Cd stress. The 10 d old *TaSFT2L* RNAi/ OE lines and WT were transferred to a nutrient solution containing different Cd concentrations (0, 0.5, 2, 10 mM Cd) for 7 d. (A - B) The Cd content of RNAi lines; (C - D) The Cd content of OE lines; Different letters (a - f) indicate significant differences ($p < 0.05$).

Similar results were observed in a chronic growth experiment with 0.5mm Cd for eight weeks. Relative to wild type, the RNAi lines revealed a better growth phenotype, and the Cd accumulation in shoots and roots was less than OE lines (Figure 4.31, Figure 4.32).

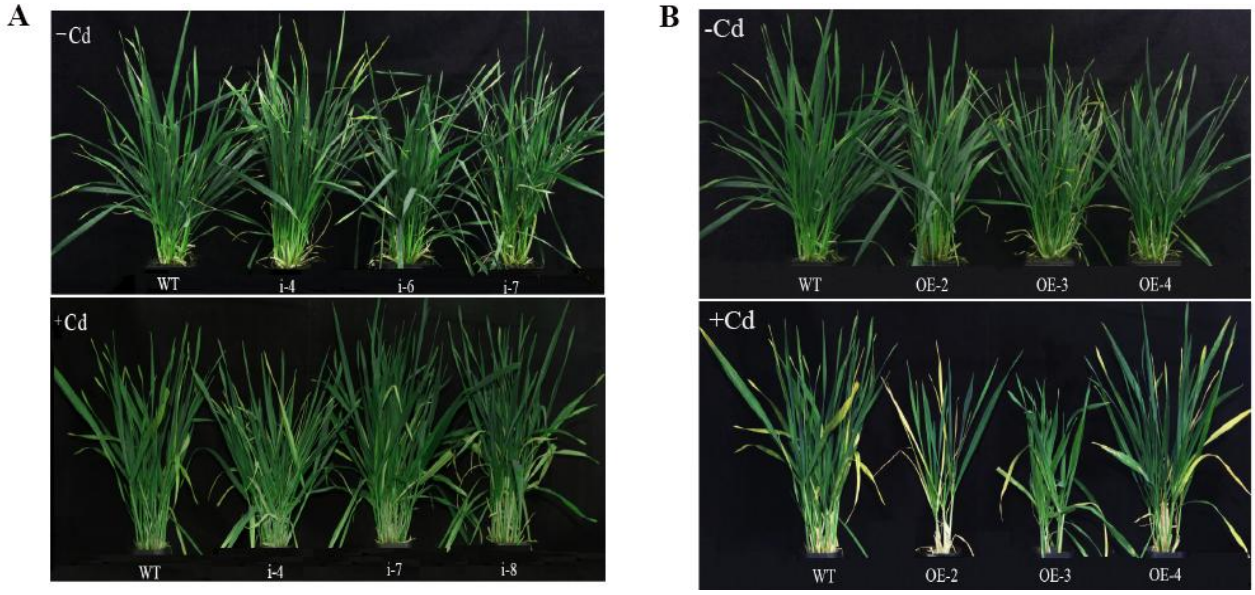

Figure 4.31. The phenotypes of wild type (WT) and transgenic plants grown in soil with 0.5 mM Cd condition for two month. (A - B) Phenotypes of transgenic lines (RNAi and OE) lines and WT under normal and 0.5 mM Cd condition.

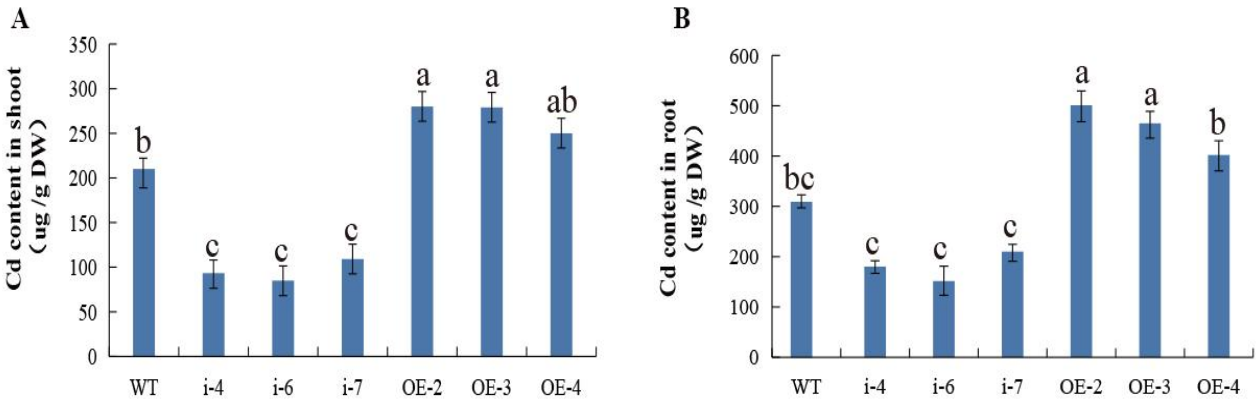

Figure 4.32. The phenotypes of wild type (WT) and transgenic plants (RNAi and OE) grown in soil with 0.5 mM Cd condition for two month. (A, B) Concents of Cd in shoots and roots. Different letters (a - f) indicate significant differences ($p < 0.05$).

(5) *TaSFT2L* gene negatively regulates wheat yield

To test whether *TaSFT2L* RNAi/OE lines suppressed Cd buildup in the grain, RNAi/OE lines were grown in a 20 mg Cd kg^{-1} - supplemented soil. By observing the mature wheat (Figure 4.33), it was found that *TaSFT2L* RNAi lines had larger ears and fuller grains than OE lines and WT plants. Grain length of wheat in *TaSFT2L* RNAi lines was significantly higher than that in OE lines and WT plants. Meanwhile, grains width of *TaSFT2L* RNAi lines was significantly higher than that of OE lines and WT plants. In addition, it could be concluded from Figure 4.33, OE lines and WT plants had small and dry grains, while *TaSFT2L* RNAi lines had large and full wheat grains. The spike number, grain number per spike, 1000 - grain weight and yield per plant of *TaSFT2L* RNAi lines were significantly higher than those of OE lines and WT plants Figure 4.34, indicating that *TaSFT2L* gene silencing promoted wheat heading and grain filling, and significantly increased wheat yield. In short, *TaSFT2L* gene was a negative regulation gene of wheat yield.

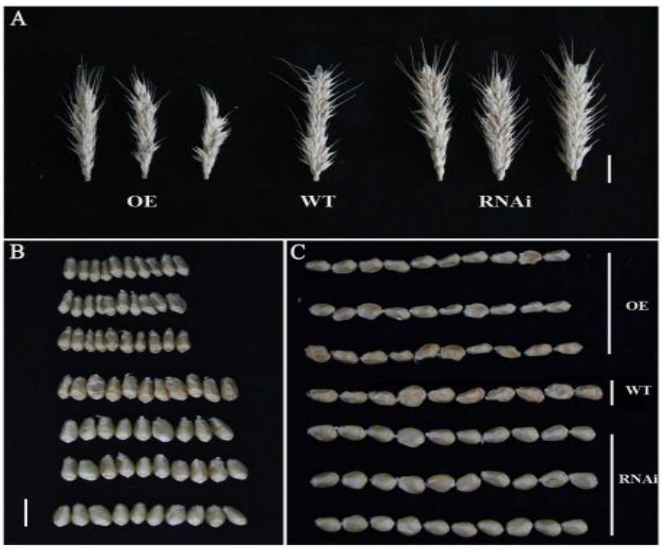

Figure 4.33. The grain physical properties of WT and transgenic plants (RNAi and OE lines) grown in 20 mg kg^{-1} Cd soil condition. (A,B,C) Phenotypes of grain.

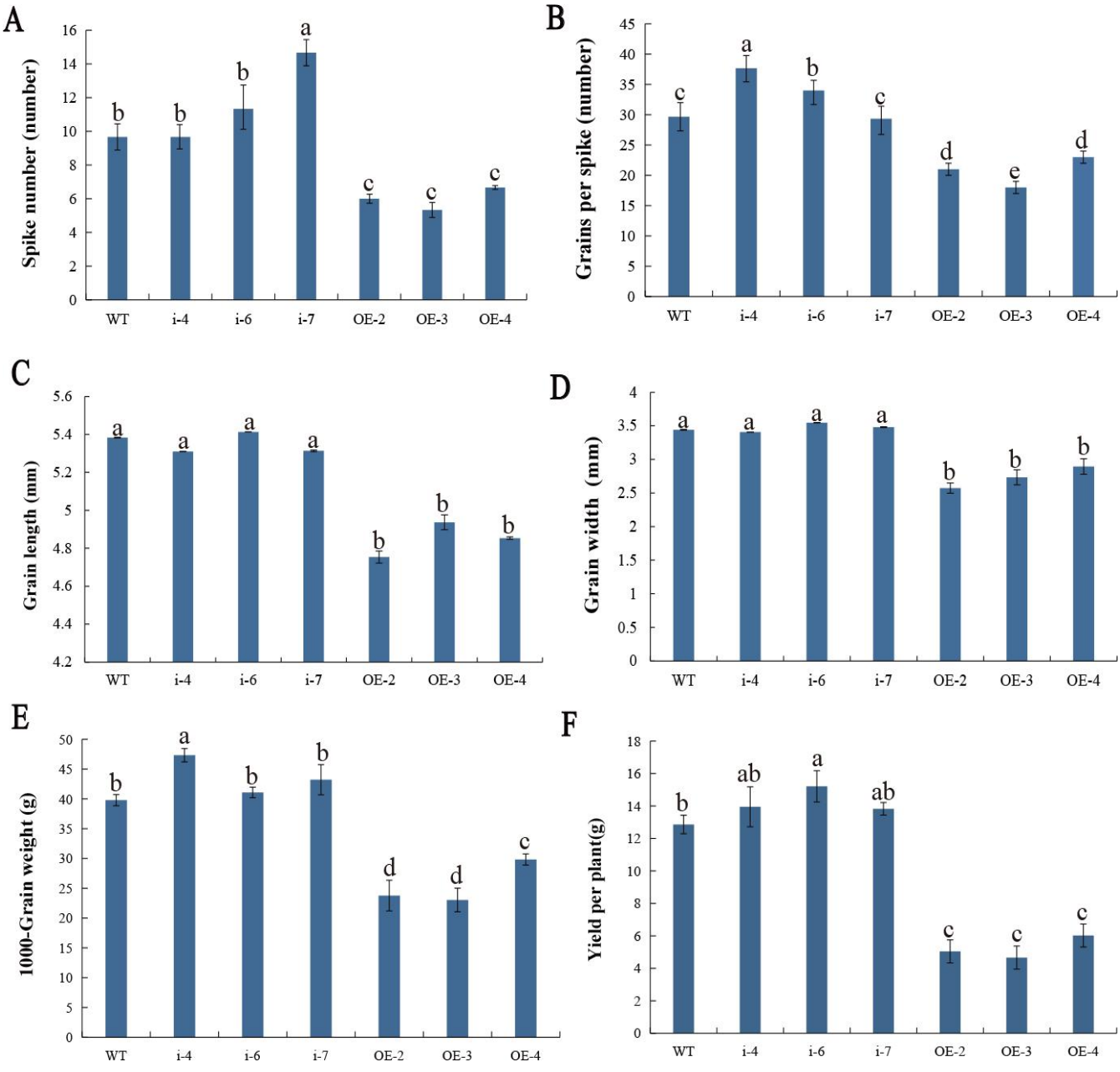

Figure 4.34. The grain physical properties of wild type (WT) and transgenic plants (RNAi and OE lines) grown in 20 mg kg^{-1} Cd soil condition. (A - F) yield and its composition; Different letters (a - f) indicate significant differences ($p < 0.05$).

(6) The silencing of *TaSFT2L* accumulated less Cd in wheat straw and grain

Grains as well as straws from major stems were evaluated for Cd concentrations. It was found that RNAi lines had significantly lower levels of Cd in wheat straw, particularly in the mature grains in which the Cd content was reduced by 29.40 - 74.95% compared to those of wild - type (Figure 4.35A). Importantly, the OE lines accumulated more Cd in the tissues than in the wild type (Figure 4.35B). Overall, these findings indicate that silencing of *TaSFT2L* could prevent Cd accumulation in wheat plants.

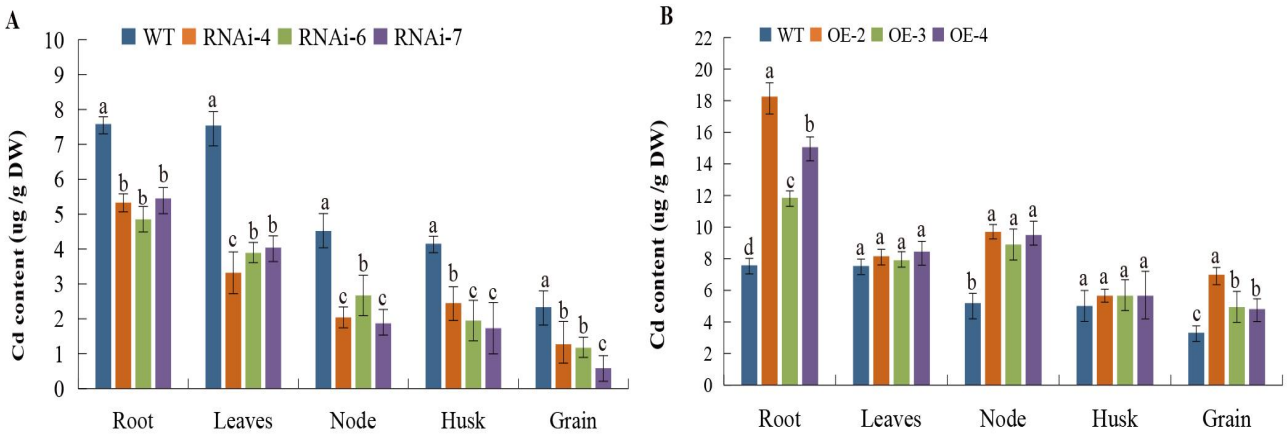

Figure 4.35. The grain physical properties of wild type (WT) and transgenic plants (RNAi and OE lines) grown in 20 mg kg^{-1} Cd soil condition. (A - B) Cd contents in wheat straws and grains; Different letters (a - f) indicate significant differences ($p < 0.05$).

Conclusions to Chapter 4

1. The *GOT1/TaSFT2* family genes encode trans - membrane transporters that play vital roles in metal exclusion, storage, immobilization and ROS detoxification. *TaSFT2L* is a crucial member of the *GOT1 / SFT2* family whose role in tolerance of a plants to heavy metals has not been fully established. In this study, the *TaSFT2L* sequence was found to be highly conserved and exhibited a high homology with other plant species. The TaSFT2L - GFP fusion protein was found to be highly localized in the cytomembrane of wheat protoplasts. Cd toxicity caused rapid Cd - induced up - regulation of *TaSFT2L* in roots, the main site of Cd toxicity in plants. This finding suggests a specific role of *TaSFT2L* in Cd tolerance.

2. *TaSFT2L* was established to be located on the cytomembrane. This is understandable since it safeguards subcellular organelles against Cd toxicity. To understand whether *TaSFT2L* can transport or detoxify metals, we leveraged yeast mutant cell models for studying fundamental processes of metal uptake and detoxification. The cellular tolerance assay showed that *ycf1 - TaSFT2L* expressions in yeast enabled the cells to tolerate Cd toxicity, as indicated by poorer growth rates under Cd stress. And the *SFT2L* mutant yeast cells accumulated numerous autophagosomes in vacuoles. This result is crucial for understanding how to limit heavy metal toxicity in plants.

3. Under heavy metal stress, inhibition of the cytomembrane proton pump in root cells may also result from alterations at transcriptional and post - transcriptional levels. However, differences in cell wall Cd levels were insignificant.

Toxic metal detoxification could be attained by several mechanisms, such as compartmentalization (vacuolar sequestration), chelation via metal ligands, including metallothioneins and phytochelatins, and toxic metal efflux from cells. Vacuoles are dominant sites for Cd ion binding, promoting the ability of plants to resist toxic metal ions, thereby protecting soluble fractions and organelles. Under same Cd concentrations, BSMV: *TaSFT2L* - inoculated plants exhibited less Cd toxicity, relative to WT. This result suggests that Cd stress can be ameliorated by Cd sequestration and/or retention in roots. Vacuolar separation of Cd - induced *TaSFT2L* silencing in plants is a Cd tolerance mechanism in wheat. We showed that BSMV: *TaSFT2L* inoculation suppressed ROS levels in roots, enhanced cell vitalities and root growth rates. Indeed, ROS detoxification occurs when antioxidants reduce ROS - mediated oxidative stress, which occur as responses to elevated cell metal levels.

4. There were strong autophagic activities in roots of BSMV: *TaSFT2L* - inoculated plants, which was substantiated by the fact that *TaSFT2L* was downregulated while autophagosomes formed more frequently under Cd treatment. Therefore, autophagy is a protective mechanism that helps plants to survive various adverse environments. After autophagic induction, a dual membrane autophagosome is formed, which transfers toxic metals to vacuoles for degradation.

5. The concentration of Cd in roots was reduced in BSMV: *TaSFT2L* - inoculated plants, which might be one of the reasons why BSMV: *TaSFT2L* - inoculated plants were more tolerant to Cd than WT, implying a relationship between autophagy and ion transport in Cd stress responses. This result of impaired

autophagy in *TaSFT2L* knockdown plants, together with complementation of yeast autophagy - defective cells with *SFT2L* demonstrates the essential role of *TaSFT2L* in wheat autophagy. Future studies should evaluate gene functions in plant responses to Cd stress.

6. Cd accumulation in transgenic lines was analyzed. Conversely, OE lines had increased Cd accumulation. This observation was consistent with the yeast experimental result, confirming that *TaSFT2L* overexpression increases toxic Cd damage in wheat, compared to WT. To enhance the accuracy of the study, a 60 day - long study with 0.5 mm Cd was conducted, and a similar result was observed: *TaSFT2L* overexpression was associated with poorer growth. Normally, these effects are random and may be associated with tissue culture procedures during transformation or insertion positions of transgenes in the genome. Furthermore, the *Atgn9* mutant (*Arabidopsis* mutant of *TaSFT2L*) exhibited tolerance to Cd.

These results support that *TaSFT2L* is a negative regulator of Cd stress. Functional identification of the *TaSFT2L* gene from wheat by the long - term soil (20 mg kg^{-1} $CdCl_2$) assay proved that reducing Cd accumulation in roots can inhibit Cd transfer to grains. *TaSFT2L* RNAi lines of wheat exhibited decreased Cd levels in roots by 28.13 - 36.04% and reduced proportions of Cd transferred from roots to grains by about 68%. Compared to the WT, OE lines exhibited increased Cd accumulation by 1.57 - 2.5 folds from the roots to the grains. Thus, our findings demonstrate that *TaSFT2L* silencing is potentially useful since it can help generate genetically modified genotype materials with low Cd accumulation in wheat grains.

CONCLUSION

The dissertation provides a theoretical generalization and a new solution to the scientific task of establishing the selection value of the source material of winter wheat, based on the trait of resistance to Cd accumulation, determining the role of individual genes in the control of this feature.

1. As a result of studying the collection samples of wheat (40 varieties) from different institution - originators, samples with valuable breeding characteristics were identified. Growth parameters such as height and leaf surface area were analyzed in the studied wheat varieties. These traits were related to productivity parameters such as 1000 seed weight, grain weight per ear, and yield.

2. The range of variation of the sign of Cd content in plants and grains of winter wheat varieties was determined: 0.91 - 2.02 and 0.06 - 2.56 mg / kg, respectively. Varieties had minimum indicators of Cd content in grain: Oktava odesk'a. Svitanok myronivskyi, Melody odes'ka, Kubok, Shchedra nyva. The maximum level of Cd content was noted in varieties of Rozkvit, Sich, Kantata odes'ka, Duma odes'ka.

3. In groups of varieties with different Cd content, differences in the structure of correlations between the main selection - controlled parameters were established. It was determined that the group of "low cadmium varieties" is characterized by the presence of direct correlations between the Cd content and parameters of vegetative development of plants, namely plant height (r=0.54) and stem weight (r=0.88). In addition, a reliable negative correlation was noted with the

indicators of the seed weight per ear (r= - 0.60) and the 1000 seed weight (r= - 0.74).

4. In the variety group with a high content of Cd, a reliable level of correlation of metal content was with the LAR indicator (r= - 0.68) and the absence of statistically significant relationships with plant productivity indicators was noted. Significant differences in the correlation structure of the Cd content trait in seeds between groups of varieties with high and low Cd content indicate a difference in the mechanisms of genetic control of this trait.

5. The distribution of inheritance frequencies of resistance trait to Cd accumulation in F1 was calculated. After cross - breing low - Cd varieties, the frequency of inheritance according to the type of heterosis is 12%, incomplete positive dominance - 8%, intermediate inheritance - 16%, and according to the type of depression and incomplete negative dominance - 44%. Inheritance according to the type of heterosis was noted in the combinations: Melody odes'ka x Ovidyi, Kubok x Svitanok myronivskyi and Shchedra nyva x Okhtyrchanka juvileyna.

6. It was established that a characteristic feature of hybrids with minimal Cd content in seeds were inverse and statistically significant correlations between the values of Cd content and indicators of the seed weight per ear (r= - 0.70), 1000 seed weight (r= - 0.78) and estimated yield (r = - 0.74). In the group of hybrids with a high level of Cd concentration in seeds, a reliable level of correlation between the values of this indicator and the controlled traits was not found.

7. Six samples were allocated for selection work: 19/1, 19/13, 19/26, 19/40, 19/33, and 19/39, obtained in combinations of Melody odesk'a x Svitanok

myronivskyi, Melody odesk'a x Shchedra nyva, Kubok x Svitanok myronivskyi, Shchedra nyva x Kubok, Zorepad x Okhtyrchanka juvileyna, Shchedra nyva x Okhtyrchanka juvileyna. The samples exceed the conventional standard in terms of Cd content in seeds, which is one of the crop structure indicators.

8. The role of individual genes in the control of processes and mechanisms of Cd accumulation in winter wheat plants was determined. A new locus *TaSFT2L*, with its novel biological functions in Cd translocation and accumulation in winter wheat plants was establisled. *TaSFT2L* - novel *GOT/SFT2s* member termed *SFT2L* was functionally characterized. The gene *TaSFT2L* plays an important role in the transport of Cd in plant.

9. The TaSFT2L sequence was found to be highly conserved and exhibited a high homology with other plant species. The TaSFT2L - GFP fusion protein was determined to be localized in the cytomembrane of wheat protoplasts. TaSFT2L gene was mainly expressed in roots and Cd toxicity caused rapid Cd - induced up - regulation of TaSFT2L there. It was observed that increased autophagic activity in roots of winter wheat caused by silencing of TaSFT2L enhanced Cd tolerance.

10. *TaSFT2L* was determined as negative regulator of Cd uptake. *TaSFT2L* gene expression in wheat has been shown to suppress Cd accumulation. It was studied *TaSFT2L* involved in tolerance to the toxic metal Cd in wheat and usually targets cell membranes. Vacuolar separation of Cd - induced *TaSFT2L* silencing in plants is a Cd tolerance mechanism in wheat.

11. The overexpression (OE) of *TaSFT2L* due to Cd toxicity resulted in compromised growth response, with higher Cd accumulation in wheat tissues. The

results proved that *TaSFT2L* was a key gene regulating Cd translocation in wheat. It was reaveld that silencing the functional gene *TaSFT2L* to form transgenic wheat can inhibit Cd accumulation in wheat grains. Functional identification of the *TaSFT2L* gene proved that reducing Cd accumulation in roots can inhibit Cd transfer to grains.

12. Transgenic wheat has been revealed that RNA interference (RNAi) lines enhanced the wheat growth concerning the increased shoot or root elongation, dry weight and chlorophyll accumulation. Furthermore, RNAi lines decreased roots to grains Cd translocation in wheat by nearly 68% and Cd accumulation in winter wheat grains by 53%.

13. It was found that *TaSFT2L* is a key gene involved in regulation of Cd translocation in winter wheat and its silencing to form transgenic wheat can inhibit Cd accumulation. *TaSFT2L* silencing gene from winter wheat was determined that it is potentially useful since it can help to generate genetically modified genotype materials with low Cd accumulation in wheat grains.

TaSFT2L silencing is potentially useful since it can help create new genotypes in breeding programs with low Cd accumulation in wheat grains. *TaSFT2L* is patened in China (Appendex E)

14. An original selection material was created, which is being researched and refined in scientific programs at Sumy National Agrarian University, the Institute of Agriculture of North-East of Ukraine and Henan Institute of Science and Technology (Xinxiang, China).

PROPOSALS FOR BREEDING

1. In the programs for creating initial material of winter wheat with a controlled level of cadmium accumulation, use the following varieties: Oktava odesk'a; Svitanok myronivskyi ; Melody odesk'a, Kubok; Shchedra nyva.

2. For targeted creation of varieties with low cadmium content and a complex of adaptive traits to the conditions of the North - Eastern Forest - Steppe of Ukraine, use selection samples 19/12; 19/13; 19/26; 19/40; 19/33; 19/39.

3. *TaSFT2L* gene from wheat can be used in developing new modified genotype materials with low Cd uptake in crop seeds.

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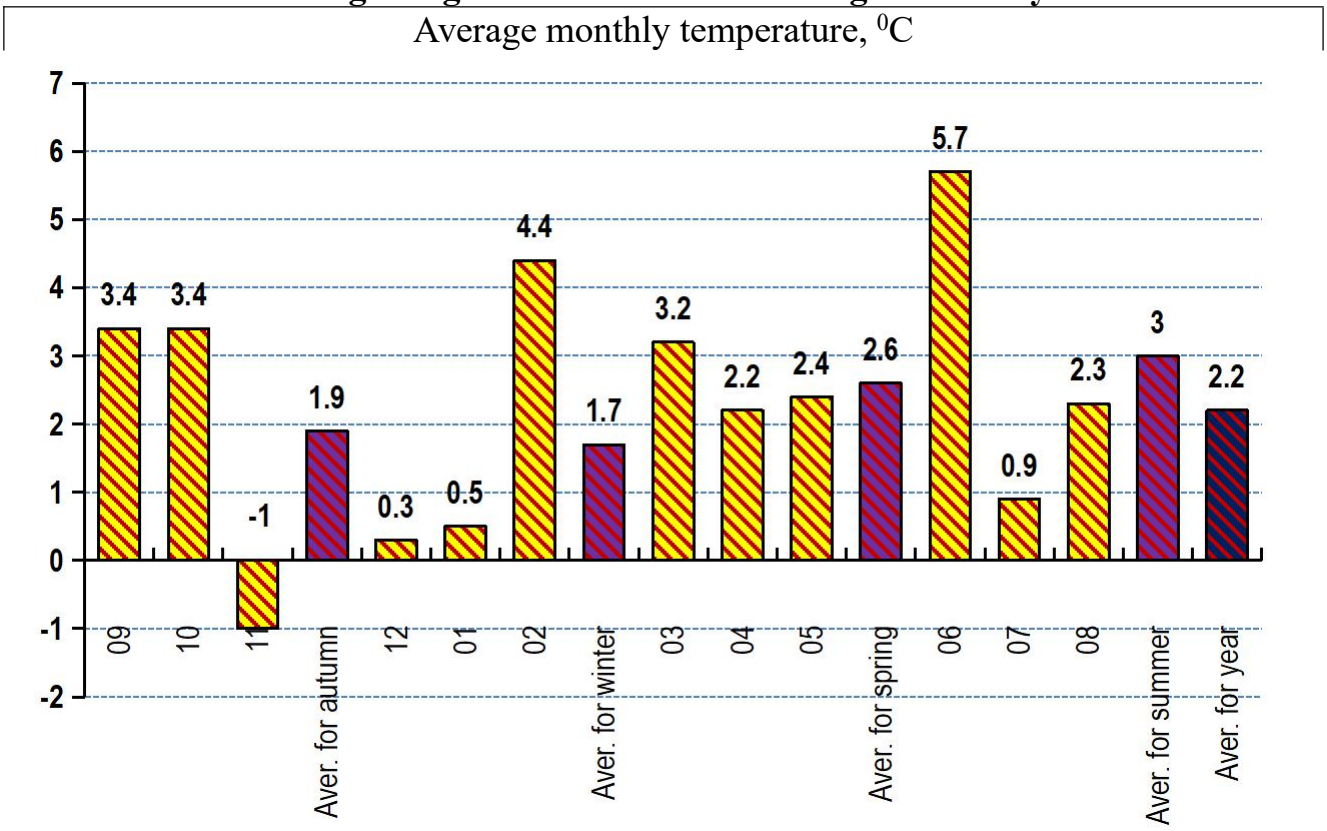
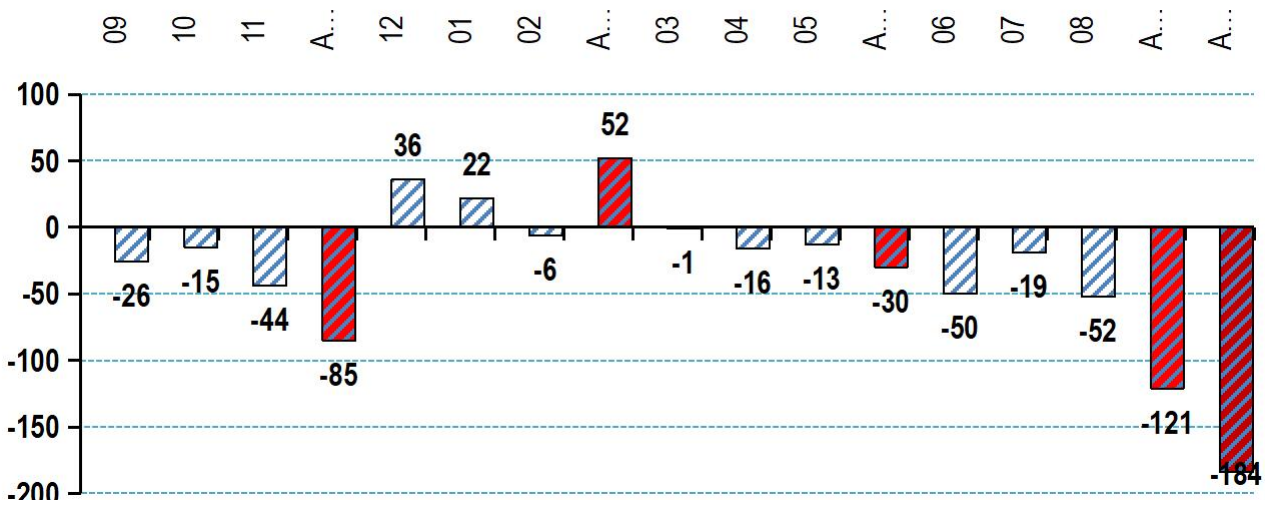
APPENDEXES

Appendix A

Dynamics of average monthly temperatures and precipitation compared to the average long-term in the 2018-2019 agricultural year



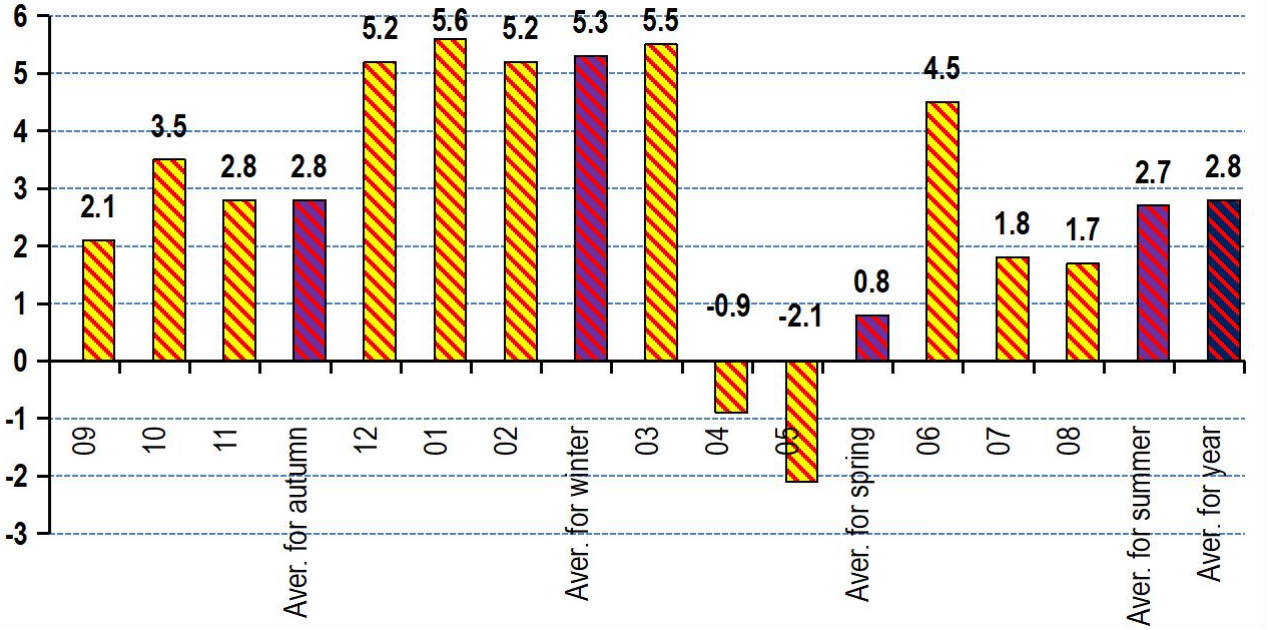
Precipitation, mm



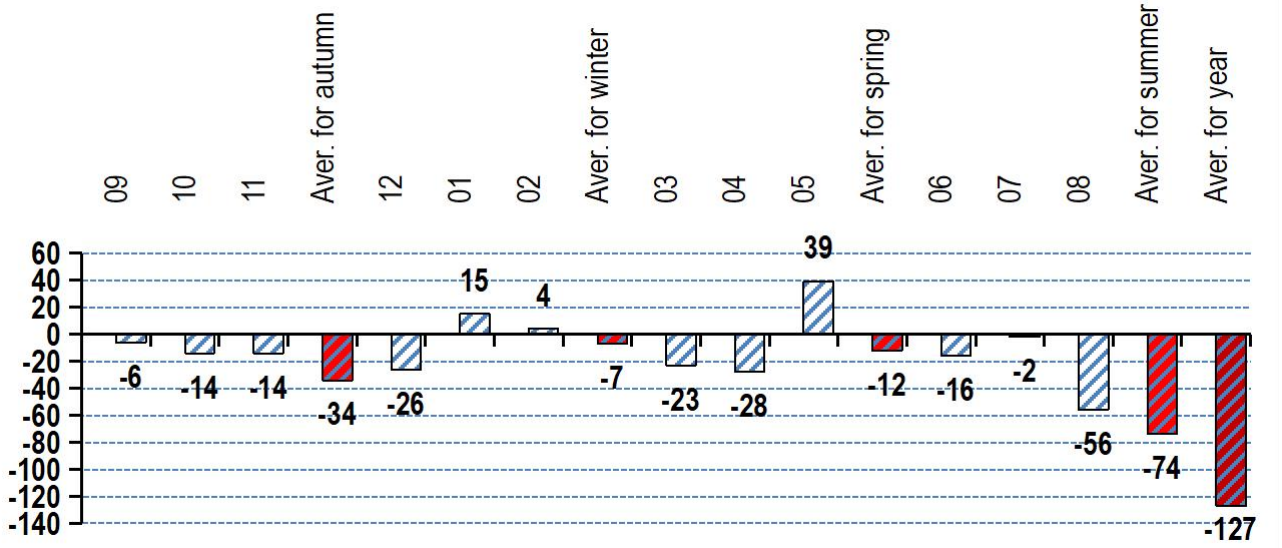
Appendix A1

Dynamics of average monthly temperatures and precipitation compared to the long-term average in the 2019-2020 agricultural year

Average monthly temperature, ^{0}C



Precipitation, mm



Appendix B

Characteristics of the collection of intervarietal hybrids according to the indicator of cadmium content in seeds (2020-2021)

№	Breeding number	origin		Cd content in grain , mg/kg
		Number of origin		
1	19/11	11/15	Sich x Duma odes'ka	0,82
2	19/12	13/12	Melody odes'ka x Svitanok myronivskyi	0,04
3	19/13	13/32	Melody odes'ka x Ovidyi	0,04
4	19/14	15/11	Duma odes'ka x Sich	0,81
5	19/15	15/24	Duma odes'ka x Kantata odes'ka	0,67
6	19/16	15/20	Duma odes'ka x Rozkvit	0,62
7	19/17	15/34	Duma odes'ka x Rusyava	0,71
8	19/18	15/20	Duma odes'ka x Rozkvit	0,91
9	19/19	21/28	Kubok x Zorepad	0,12
10	19/20	21/32	Kubok x Ovidyi	0,11
11	19/21	21/36	Kubok x Shchedra nyva	0,09
12	19/22	21/41	Kubok x Slaven	0,1
13	19/23	41/13	Slaven x Melody odes'ka	0,07
14	19/24	41/28	Slaven x Zorepad	0,1
15	19/25	41/36	Slaven x Shchedra nyva	0,08
16	19/26	21/12	Kubok x Svitanok myronivskyi	0,04
17	19/27	41/37	Slaven x Oktava odes'ka	0,06
18	19/28	12/21	Svitanok myronivskyi x Kubok	0,12
19	19/29	12/37	Svitanok myronivskyi X Oktava odes'ka	0,1
20	19/30	12/28	Svitanok myronivskyi X Zorepad	0,08
21	19/31	32/28	Ovidyi x Zorepad	0,42
22	19/32	13/10	Melody odes'ka x Okhtyrchanka juvileina	0,14
23	19/33	28/10	Zorepad x Okhtyrchanka juvileina	0,06
24	19/34	13/12	Melody odes'ka x Svitanok myronivskyi	0,21
25	19/35	13/21	Melody odes'ka x Kubok	0,1

26	19/36	13/36	Melody odes'ka x Shchedra nyva	0,34
27	19/37	28/10	Zorepad x Okhtyrchanka juvileina	0,23
28	19/38	28/13	Zorepad x Melody odes'ka	0,34
29	19/39	36/10	Shchedra nyva x Okhtyrchanka juvileina	0,06
30	19/40	36/21	Shchedra nyva x Kubok	0,05
31	19/41	10/21	Okhtyrchanka juvileina x Kubok	0,24

Appendix C

Correlations matrix of the main variety parameters of working collection of winter wheat (2018 - 2021)

Trait	Height, cm	Weight of stem, g	Leaf area, m^2		LAI, m^2/m^2	Chlorophyll, мг/г	Crop density, spikelets/$м^2$	Yield, t/ha	Seed weight per ear , g	1000 seed weight, g	Seed number in ear, pcs	LAR, m^2/kg
Height, cm		0,67	0,34		0,20	-0,24	-0,23	0,18	0,32	0,29	0,15	0,18
Weight of stem, g	0,67		0,78		0,55	0,09	-0,35	0,21	0,46	0,50	0,15	0,52
Leaf area, m^2	0,34	0,78			0,88	0,16	-0,08	0,12	0,12	0,24	-0,06	0,88
LAI, m^2/m^2	0,20	0,55	0,88			0,04	0,37	0,30	-0,22	-0,09	-0,19	0,96
Chlorophyll, mg/г	0,24	0,09	0,16		0,04		-0,14	-0,10	0,07	0,21	-0,07	0,07
Crop density, spikelets/m^2	-0,23	-0,35	-0,08		0,37	-0,14		0,32	-0,81	-0,66	-0,46	0,30
Yield, t/ha	0,18	0,21	0,12		0,30	-0,10	0,32		0,27	0,01	0,36	0,02
Seed weight per ear, g	0,32	0,46	0,12		-0,22	0,07	-0,81	0,27		0,66	0,71	-0,30
1000 seed weight, g	0,29	0,50	0,24		-0,09	0,21	-0,66	0,01	0,66		-0,06	-0,09
Seed number per ear, pcs	0,15	0,15	-0,06		-0,19	-0,07	-0,46	0,36	0,71	-0,06		-0,31
LAR, m^2/kg	0,18	0,52	0,88		0,96	0,07	0,30	0,02	-0,30	-0,09	-0,31	

Appendix C1

Correlation matrix of main parameters of winter wheat variety group resistant to cadmium accumulation (2019 – 2021)

Trait	Cd content, mg/kg,	Height, cm	Stem weight, g	Leaf area, м²a 2	LAI, m²/m²	Chlorophyll, mg/g	Crop density, spikelets/m	Yield, t/ha	Seed weight per ear, g	1000 seed weight, g	Seed number in ear, pcs	LAR, m²/kg,
Cd content, mg/kg		0,549	0,885	0,487	0,305	0,295	-0,336	0,344	-0,603	-0,738	-0,021	0,199
Height, cm	**0,549**		0,260	-0,452	-0,457	-0,413	0,189	0,114	-0,097	-0,181	0,142	-0,538
Stem weight, g	0,885	0,260		0,628	0,393	0,403	-0,460	0,361	0,731	0,804	-0,195	0,277
Leaf area, м²	0,487	-0,452	0,628		0,834	0,717	-0,525	0,268	0,733	0,739	-0,081	0,800
LAI, m²/m²	0,305	-0,457	0,393	0,834		0,416	-0,012	0,498	0,370	0,375	-0,081	0,852
Chlorophyll, mg/g	0,295	-0,413	0,403	0,717	0,416		-0,500	-0,054	0,513	0,747	-0,408	0,505
Crop density, spikelets/m²	-0,336	0,189	-0,460	-0,525	-0,012	-0,500		0,304	-0,782	-0,719	-0,098	-0,197
Yield, t/ha	0,344	0,114	0,361	0,268	0,498	-0,054	0,304		0,346	0,109	0,320	-0,026
Seed weight per ear, g	-0,603	-0,097	0,731	0,733	0,370	0,513	-0,782	0,346		0,810	0,258	0,225
1000 seed weight, g	-0,738	-0,181	0,804	0,739	0,375	0,747	-0,719	0,109	0,810		-0,355	0,383
Seed number in ear, pcs	-0,021	0,142	-0,195	-0,081	-0,081	-0,408	-0,098	0,320	0,258	-0,355		-0,302
LAR, m²/kg	0,199	-0,538	0,277	0,800	0,852	0,505	-0,197	-0,026	0,225	0,383	-0,302	

Appendix C2

Correlation matrix of the main parameters main parameters of winter wheat variety group with a high level of cadmium accumulation (2018 – 2021)

Trait	Cd content, mg/kg,	Height, cm	Stem weight, g	Leaf area, м²а 2	LAI, m²/m²	Chlorophyll, mg/g	Crop density, spikelets/m	Yield, t/ha	Seed weight per ear, g	1000 seed weight, g	Seed number in ear, pcs	LAR, m²/kg,
Cd content, mg/kg		-0,421	-0,495	-0,540	-0,625	0,288	0,063	0,177	-0,076	-0,066	-0,026	-0,686
Height, cm	-0,421		0,987	0,976	0,922	-0,627	-0,775	0,480	0,897	0,919	0,392	0,910
Stem weight, g	-0,495	0,987		0,997	0,969	-0,707	-0,668	0,484	0,819	0,882	0,275	0,960
Leaf area, м²	-0,540	0,976	0,997		0,984	-0,705	-0,623	0,489	0,785	0,847	0,259	0,976
LAI, m²/m²	-0,625	0,922	0,969	0,984		-0,719	-0,478	0,491	0,672	0,750	0,170	0,994
Chlorophyll, mg/g	0,288	-0,627	-0,707	-0,705	-0,719		0,205	-0,210	-0,336	-0,665	0,464	-0,730
Crop density, spikelets/m²	0,063	-0,775	-0,668	-0,623	-0,478	0,205		-0,195	-0,927	-0,822	-0,657	-0,473
Yield, t/ha	0,177	0,480	0,484	0,489	0,491	-0,210	-0,195		0,532	0,488	0,362	0,394
Seed weight per ear, g	-0,076	0,897	0,819	0,785	0,672	-0,336	-0,927	0,532		0,917	0,654	0,637
1000 seed weight, g	-0,066	0,919	0,882	0,847	0,750	-0,665	-0,822	0,488	0,917		0,298	0,724
Seed number in ear, pcs	-0,026	0,392	0,275	0,259	0,170	0,464	-0,657	0,362	0,654	0,298		0,132
LAR, m²/kg	-0,686	0,910	0,960	0,976	0,994	-0,730	-0,473	0,394	0,637	0,724	0,132	

Appendix C3

CHARACTERISTICS OF CREATED HYBRIDS

Breeding sample	Origin	Cadmium content in grain, mg / kg	Plant height, cm	Grain number per ear, pcs	Grain weight per ear, (g)	Weight of 1000, g	Calculated productive density, ears / m^2*	Calculated crop capacity t / h a **
Breeding samples with a low level of cadmium accumulation								
19/12	Melody o des ' ka x Svitanok myronivskyi	0,04	61,10	26,80	0,95	35,45	480,1	4,33
19/13	Melody o des ' ka x Shchedra Nyva	0,04	68,16	30,02	1,37	45,47	465,1	6,03
19/26	Kubok x Svitanok myronivskyi	0,04	87,18	30,17	1,46	48,40	435,2	6,04
19/40	Shchedra Nyva x Kubok	0,05	62,64	24,05	1,14	47,40	401,5	4,35
19/33	Zorepad x O khtyrchanka Juvileina	0,06	56,13	43,73	1,20	27,44	380,7	4,34
19/39	Shchedra Nyva x O khtyrchanka Juvileina	0,06	70,70	24,53	1,10	44,84	410,6	4,29
Breeding samples with a high level of cadmium accumulation								
19/11	Sich x O khtyrchanka Juvileina	0,82	76,10	26,79	1,02	38,07	376,7	3,65
19/18	Duma o des' ka x O khtyrchanka Juvileina	0,91	72,93	31,34	1,15	36,70	394,9	4,31

*-for sowing rate, 67 grains of linear meter
**- calculated from a plot of 1.5 linear meter

Appendix C4

Correlations matrix of the productivity parameters of winter wheat hybrids with low Cd accumulation

Показник	Cd content in seed, mg/kg	Height, cm	Height, cm	Seed nunber per ear, pcs	Seed weight per ear (g)	1000 seed weight, g	Estimated productive density, ear /m^2	Estimated yield, t/ha
Cd content in seed, mg/kg		0,395593	0,246172	0,499956	-0,703728	-0,788271	-0,152722	-0,739859
Height, cm	0,395593		0,498364	0,098646	-0,066746	-0,133787	0,047136	-0,041887
Ear lengh, cm	0,246172	0,498364		0,317785	-0,192888	-0,371185	-0,090575	-0,191579
Seed nunber per ear, pcs	0,499956	0,098646	0,317785		-0,137790	-0,684675	-0,402004	-0,300562
Seed weight per ear (g)	-0,703728	-0,066746	-0,192888	-0,137790		0,810648	-0,032282	0,920557
1000 seed weight, g	-0,788271	-0,133787	-0,371185	-0,684675	0,810648		0,173076	0,830450
Estimated productive density, ear /m^2	-0,152722	0,047136	-0,090575	-0,402004	-0,032282	0,173076		0,354900
Estimated yield, t/ha	-0,739859	-0,041887	-0,191579	-0,300562	0,920557	0,830450	0,354900	

Appendix C5

Correlations matrix of the productivity parameters of winter wheat hybrids with high Cd accumulation

Trait	Cd content in seed, mg/kg	Height, cm	Height, cm	Seed number per ear, pcs	Seed weight per ear (g)	1000 seed weight, g	Estimated productive density, ear /m^2	Estimated yield, t/ha
Cd content in seed, mg/kg		0,266422	0,310355	-0,443465	0,574585	0,765442	0,284674	0,718257
Height, cm	0,266422		-0,003966	0,471156	0,488947	0,246260	-0,819928	0,248736
Ear lengh, cm	0,310355	-0,003966		-0,194429	-0,259201	-0,154931	0,247405	-0,153863
Seed number per ear, pcs	-0,443465	0,471156	-0,194429		0,196982	-0,361390	-0,838588	-0,080213
Seed weight per ear (g)	0,574585	0,488947	-0,259201	0,196982		0,839005	-0,286287	0,945691
1000 seed weight, g	0,765442	0,246260	-0,154931	-0,361390	0,839005		0,148794	0,925396
Estimated productive density, ear /m^2	0,284674	-0,819928	0,247405	-0,838588	-0,286287	0,148794		0,038390
Estimated yield, t/ha	0,718257	0,248736	-0,153863	-0,080213	0,945691	0,925396	0,038390	

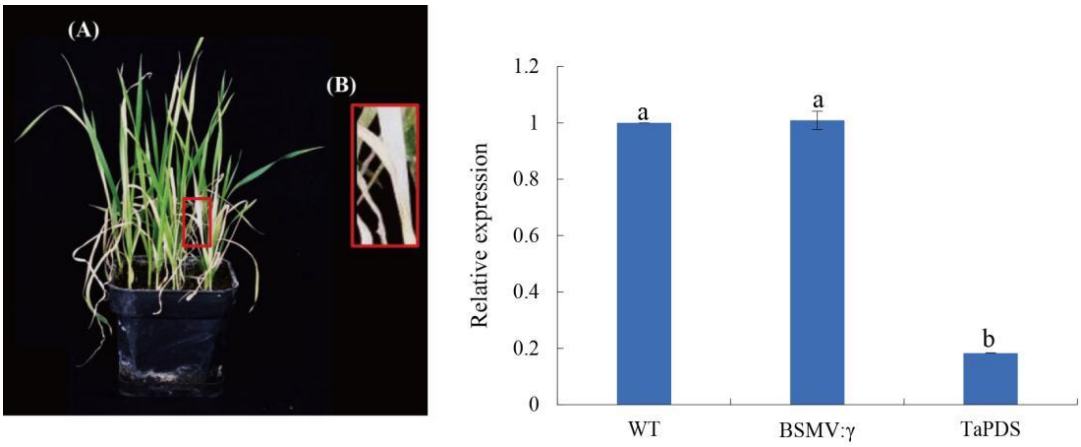
Appendix D

Primer sets used in this study

Name	Sequence (5'-3')*	Application
NOS-60	CAACAGGATTCAATCTTAAGAAAC	Cloning *Arabidopsis thaliana* mutant strains (Bu et al., 2019)
IntronFw2	CATATTGCAAAGTCTGAAGACTC	
AtSFTL-F	AACCACTGATCGTCGGTATTC	Cloning *AtSFTL*
AtSFTL-R	AATGTATCCACCAAGCAACTCT	
TaSFT2L-F	GAAGACACATGCACGAGGTT	Cloning *TaSFT2L*
TaSFT2L-R	ACACAGGCTGAGACAGATGG	
pGFP-TaSFT2L-F	CAGATCTATGCAGGCGTGGTTCTCC	Cloning Subcellular localization construct pGFP-TaSFT2L
pGFP-TaSFT2L-R	GACTAGTTCGCCCGAAGCATCT	
pYes-TaSFT2L-F	CGGGATCCATGCAGGCGTGGTTCTCC	Cloning heterologous expression construct pYes-TaSFT2L
pYes-TaSFT2L-R	CGGAATTCTCGCCCGAAGCATCT	
qTaSFTL-F	GGAGGATTCGGGATCGACAT	qPCR of *TaSFT2L*
qTaSFTL-R	TTTGCCAGATGGAACACTGC	
BMSV-TaSFTL-F	TAGCTGAGCGGCCGCCCCGGGAGTCC TCAACAAGCAGTG	Cloning VIGS construct BSMV: TaSFT2L
BMSV-TaSFTL-R	TAGCTGATTAATTAACCCGGGGAAGA CAAGAGAAGAGAACAG	
RNAi-TaSFTL-F-1	GGGTACC CCAGTCCTCAACAAGCAGTG	Cloning silencing construct RNAi-TaSFT2L
RNAi-TaSFTL-R-1	CGGGATCCCGGAGCACCATTGACACA TAGAT	
RNAi-TaSFTL-R-2	GGAGCTCGGAGCACCATTGACACATA GAT	Cloning silencing construct RNAi-TaSFT2L
RNAi-TaSFTL-R-2	CGACTAGT CCAGTCCTCAACAAGCAGTG	
OE-TaSFTL-F	GAGAACACGGGGGACTCTAGAATGCA GGCGTGGTTCTCC	Cloning overexpression construct OE-TaSFT2L
OE-TaSFTL-R	GAGCTCGGTACCCGGGGATCCTCGCC CGAAGCATCT	
TaAlpha-tubulin-F	ATCTCCAACTCCACCAGTGTCG	qPCR (Xiang et al., 2011)
TaAlpha-tubulin-R	TCATCGCCCTCATCACCGTC	
AtUBQ10-F	GATCTTTGCCGGAAAACAATTGG	qPCR
AtUBQ10-R	TAGAAAGAAAGAGATAACAGG	

Appendix D1

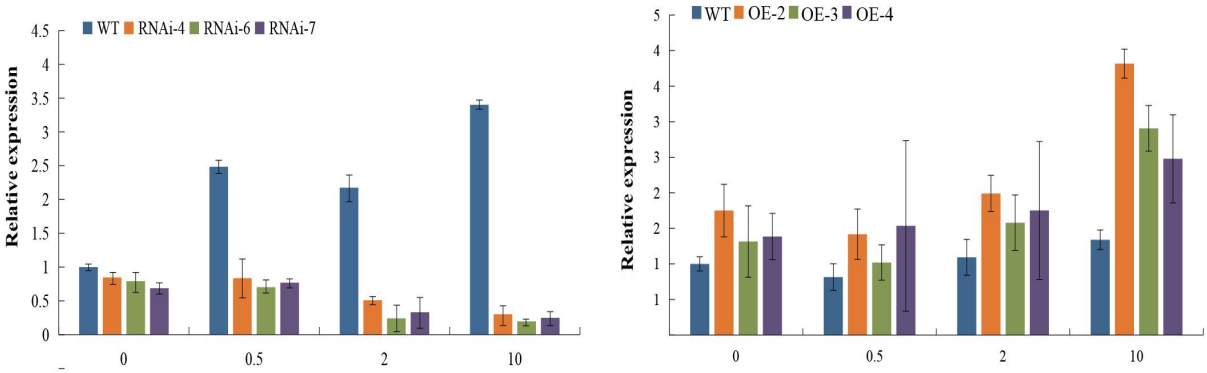
Phenotype of the BSMV: TaPDS-inoculated plants and the relative expression.



BSMV: *TaPDS*-inoculated plants and relative expression. Statistical comparison was performed by Duncan's test ($P < 0.05$).

Appendix D2

The expression level of TaSFT2L in different concentrations of Cd.



The expression level of TaSFT2L in different concentrations (0, 0.5, 2, 10mM) of Cd. Statistical comparison was performed by Duncan’ s test ($P < 0.05$).

Appendix E

Certificate No. 4999902 QR Code

Certificate of Invention Patent

Title of Invention: A wheat *TaSFT2-like* (*SFT2L*) gene in enhancing cadmium tolerance after silencing
Inventor: Yu Yongang, Wu LiuLiu, Li Chengwei, Hu Haiyan, Tao Ye
Patent No.: ZL 2020 1 0865449. X
Patent application date: August 25, 2020
Patentee: Henan Institute of Science and Technology
Address: 453000 East Section of Hualan Avenue, Hongqi District, Xinxiang City, Henan Province
Date of authorization proclamation: March 15, 2022
Authorization proclamation No.: CN 111944030 B

The China National Intellectual Property Administration has examined the invention pursuant to Patent Law of the People's Republic of China, and decided to grant it Certificate of Invention Patent and registered it in the patent register. The patent right will become effective as of the date of approval. The valid period of the patent shall be 20 years from the date of application.

The legal status when patent right is registered is recorded in the Certificate of Patent. The transfer, pledge, invalidity, termination and recovery of patent right and the information such as change of patentee's name, nationality or address etc. is recorded in the Patent Register.

Director-General: Shen Changyu

China National Intellectual Property Administration (seal)

March 15, 2022

Page 1 (Total 2 Pages)

For other matters, see continuation

Appendix E

国 家 知 识 产 权 局

发文日：

2020年8月25日

申请号或专利号：ZL 2020 1 0865449. X　　发文序号：**202082501480260**

专 利 申 请 受 理 通 知 书

根据专利法第 28 条及其实施细则第 38 条、第 39 条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：ZL 2020 1 0865449. X

申请日：2020 年 8 月 25 日

申请人：于永昂，毋柳柳，李成伟，胡海燕，陶烨

发明创造名称：一种小麦 *TaSFT2-like (SFT2L)*基因在沉默后增强镉耐受性中的应用

经核实，国家知识产权局确认收到文件如下：

说明书附图 每份页数:4 页 文件份数:1 份

发明专利请求书 每份页数:6 页 文件份数:1 份

说明书摘要 每份页数:1 页 文件份数:1 份

说明书 每份页数:8 页 文件份数:1 份

专利代理委托书 每份页数:2 页 文件份数:1 份

权利要求书 每份页数:3 页 文件份数:1 份 权利要求项数： 8 项

提示：

1. 申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应内容不一致时，可以向国家知识产权局请求更正。

2. 申请人收到专利申请受理通知书之后，再向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。

3. 国家知识产权局收到向外国申请专利保密审查请求书后，依据专利法实施细则第 9 条予以审查。

审 查 员：自动受理　　审查部门：专利局初审及流程管理部

国家知识产权局 专利审查业务章

200101
2019.11

纸件申请，回函请寄：100088 北京市海淀区蔚门桥西土城路 6 号 国家知识产权局受理处收
电子申请，应当通过电子专利申请系统以电子文件形式提交相关文件。除另有规定外，以纸件等其他形式提交的文件视为未提交。

Appendix E

З А Т В Е Р Д Ж У Ю

Проректор з науково-педагогічної
та навчальної роботи
Д. б. н., професор
……. І. М. Коваленко

Д О В І Д К А

про впровадження результатів наукових досліджень у навчальному процесі

Видана **Ву Люлю** у тому, що матеріали дисертаційної роботи «Створення вихідного матеріалу пшениці озимої стійкого до накопичення кадмію», які опубліковані у статтях:

- **Wu Liuliu**, Zhatova Halyna (2020) Basis for the breeding of low - Cd wheat varieties. Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology", 1 (39), 78–86. https://snaubulletin.com.ua/indexphp/ ab/article/.view/367/325.
- **Wu Liuliu,** Zhatova Halyna (2020) Cloning and bioinformatics analysis of cadmium resistant gene TASFT2 in wheat. Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology", 3 (41), 2020, 63-68. https://snaubulletin.com.ua/index.php/ab/article/view/379/337.
- **Wu Liuliu**, Zhatova Halyna. (2022) Study of winter wheat collection for developing initial material with low Cd - uptake Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology", 1 (47), 3-10. DOI https://doi.org/10.32845/agrobio.2022.1.1.
- **Liuliu Wu,** Yongang Yu, Haiyan Hu, Ye Tao, Puwen Song, Dongxiao Li, Yuanyuan Guan, Huanting Gao, Xiaotian Sui, Trotsenko Volodymyr, Vlasenko Volodymyr, Halyna Zhatova and Chengwei Li (2022). A New Vesicle Transport Protein SFT2 - like (SFT2L) Enhances cadmium tolerance and reduces cadmium accumulation in common wheat grains. Journal of Agricultural and Food Chemistry. DIO: https://doi.org/10.1021/acs.jafc.1c08021

включені до навчальних програм (силабусів) дисциплін «Технічні культури» і «Селекція і насінництво польових культур» та використовуються в навчальному процесі підготовки фахівців ОС «бакалавр» спеціальності 201 «Агрономія»

Довідка видана для подання до спеціалізованої вченої ради

Завідувач кафедри
агротехнологій та ґрунтознавства
д. с. – г. наук, професор
…… В. І. Троценко

Завідувач кафедри
селекції та насінництва
ім. професора М. Д. Гончарова
к. с. – г. наук, доцент
…… В. І. Оничко

Appendix E

11	19/21	Kubok x Shchedra nyva	0,09	30
12	19/22	Kubok x Slaven	0,1	30
13	19/23	Slaven x Melody odes'ka	0,07	50
14	19/24	Slaven x Zorepad	0,1	50
15	19/25	Slaven x Shchedra nyva	0,08	50
16	19/26	Kubok x Svitanok myronivskyi	0,04	30
17	19/27	Slaven x Oktava odes'ka	0,06	50
18	19/28	Svitanok myronivskyi x Kubok	0,12	50
19	19/29	Svitanok myronivskyi X Oktava odes'ka	0,1	50
20	19/30	Svitanok myronivskyi X Zorepad	0,08	50
21	19/31	Ovidyi x Zorepad	0,42	50
22	19/32	Melody odes'ka x Okhtyrchanka juvileina	0,14	50
23	19/33	Zorepad x Okhtyrchanka juvileina	0,06	50
24	19/34	Melody odes'ka x Svitanok myronivskyi	0,21	50
25	19/35	Melody odes'ka x Kubok	0,1	50
26	19/36	Melody odes'ka x Shchedra nyva	0,34	50
27	19/37	Zorepad x Okhtyrchanka juvileina	0,23	50
28	19/38	Zorepad x Melody odes'ka	0,34	50
29	19/39	Shchedra nyva x Okhtyrchanka juvileina	0,06	50
30	19/40	Shchedra nyva x Kubok	0,05	50
31	19/41	Okhtyrchanka juvileina x Kubok	0,24	50

*- *- *середнє за 2020 та 2021 роки на фоні 0,21 мг/кг*

Усього 31 (тридцять один) міжсортовий гібрид.

Завідувач кафедри агротехнологій та ґрунтознавства Сумського НАУ

…… В. І. Троценко

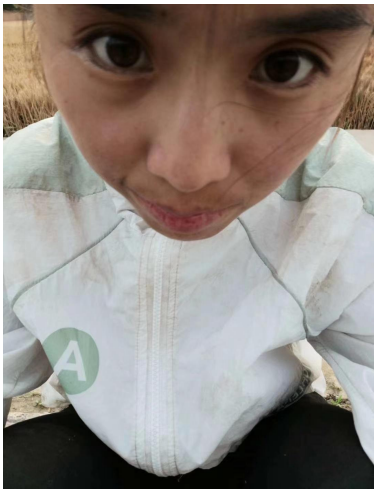
Завідувач відділу селекції та насінництва Інституту СГПС НААН

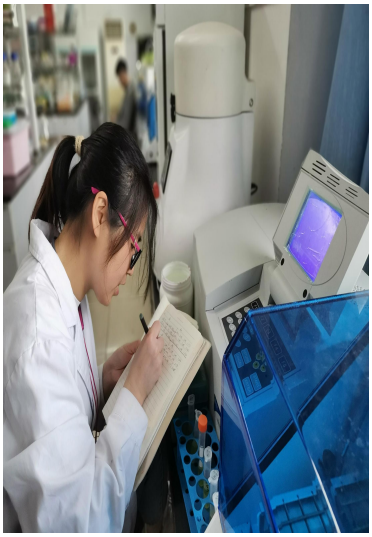
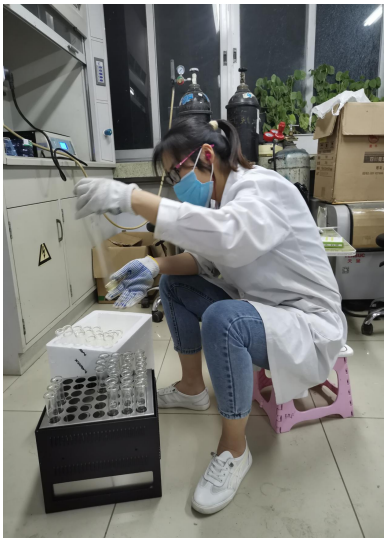
…… В. В. Кабанець

Appendix F







Appendix F