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THESIS

SELECTION OF SUNFLOWER FOR RESISTANCE TO CADMIUM ACCUMULATION

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Sunflower is the main oil crop in Ukraine, and it has a dominant influence on the formation of the world market of edible oils. According to EU standards, the content of cadmium in sunflower oil should not exceed 0,05 mg/kg. One of the ways to ensure regulatory indicators in the conditions of increasing levels of cadmium pollution is the use of sunflower varieties with a low level of accumulation of this metal. The use of such varieties can effectively reduce the content of cadmium in seeds, fundamentally solving the problem of its entry into the human body through sunflower processing products. In the research, selection samples of sunflower were screened for resistance to cadmium accumulation. Physiological and transcriptomic mechanisms of Cd accumulation in individual parts of sunflower plants were studied. As a test material, 104 selected sunflower samples were used in the experiment. Plant biomass and cadmium content were used as screening indicators in pot culture, hydroponics and field conditions. Varieties with a significant difference in cadmium accumulation were selected and experimental materials were obtained to study the mechanisms of cadmium accumulation in different parts of plants.

According to the results of a vegetation experiment using an analytical background with cadmium concentration of 1,0 mg/kg, it was established that the values indicator range of this metal concentration in the above-ground part of plants varied from 0,5 to 2,6 mg. / kg. Within this range, five groups with different levels of resistance to cadmium accumulation were distinguished.

High resistance to the accumulation of cadmium in the aerial part of plants (0,5-0,9 mg/kg) was noted in 11 samples: JB231AC, S2K670, UE0100938, IPS\125, 64\3, Polaris CL, Reason, 56\3, Snk630 , UE0100977 and UE0100018. The share of this group in the collection was 10,6%. The group with a very low level of resistance (cadmium content more than 2,11 mg/kg) included 7 selection samples PR63LL01,

UE0100056, UE0100715, 62\3, UE0100052, JG3, UE0100060. The share of this group in the collection was 6,7%.

The next stage of the work was to determine the plant resistance to the Cd accumulation in seeds (95 samples). The research was conducted under the conditions of field experiment with a natural background of 0,21 mg/kg cadmium in the soil. According to the results of the 3-year experiment, the range of the cadmium content indicator ranged from 0,23 to 0,43 mg/kg. The average value of this indicator in the seeds for the collection was 0,34±0,01 mg/kg. The coefficient of variation was 12,12%.

Comparison of data from vegetation and field experiments showed the similarity of the results regarding the differentiation of the collection by the level of resistance to cadmium accumulation. More than half, namely 63,8% of breeding samples analyzed by the method of indirect evaluation corresponded to the indicators obtained in the field experiment. The highest proportion of common (for both experiments) samples was noted in groups with low and unsatisfactory levels of Cd resistance. A group with a high level of resistance to cadmium accumulation was also characterized by a high share of joint hybrids, namely 76,1%. The frequency of joint hybrids in groups with good and satisfactory levels of resistance was 40,6 and 42,8%, respectively. In cases of "group mismatch", a shift of the selection sample to the next (higher or lower) group was observed. Therefore, the use of the method of indirect assessment ensures a high efficiency of negative selection (against traits). This method can be used to exclude samples with a low level of resistance to cadmium accumulation from a breeding program.

A detailed analysis of the results of collection differentiation using different research methods indicates that the high proportion of common (for both experiments) samples in the groups with low and unsatisfactory resistance may be the result of the universality of genetic mechanisms of a high level of cadmium accumulation in vegetative organs and sunflower seeds.

According to the results of the study of the basic collection in the conditions of the field experiment, the average values and the range of variation of the main selectively controlled traits were determined. The average value of the indicator of the duration of the "seedling-flowering" period was 56,5 days with a range of variability from 50 to 66 days. The coefficient of variation was 6,81%. The average value, range of variability, and variation coefficient of the base collection for plant productivity were 33,6 g, 11,70–63,30 g, and 20,75%, respectively.

According to the results of the cluster analysis, morphological heterogeneity was established in the group of low-cadmium samples with the selection of three intragroup clusters. The first cluster included late-ripening (the duration of the "seedling-flowering" phase > 64 days), tall (> 193 cm) and low-yielding samples UE0100114, UE01000114, UE0100977, UE0100018. The second cluster included samples with an average and higher level of plant productivity (22,6 – 52,0 g/plant). Samples with specific characteristics formed a separate cluster. The heterogeneity of the group of low-cadmium samples implies the presence of different mechanisms for the formation of seed productivity and the realization of the trait of resistance to cadmium accumulation. On the contrary, the homogeneity of the group with a "high level of cadmium accumulation" may indicate the presence of a single mechanism of transport of biologically active cadmium from vegetative organs to seeds.

The heterogeneity of the basic collection of breeding samples and the difference in the ensuring mechanisms of low level of cadmium accumulation in sunflower seeds were confirmed by the results of intervarietal crossings. 73 samples of intervarietal hybrids in F_1 and F_2 were obtained and studied. The following result was established: when crossing "low-cadmium" parental forms, only a fifth or 19% of them inherited the trait of resistance to cadmium accumulation by heterosis type. The proportions of hybrids that inherited the trait according to the type of partial positive dominance and the intermediate type for parental forms were 9,5% each. In most cases, 62% of the trait inheritance occurred according to the type of depression and partial negative dominance. Depression and partial negative dominance were also the predominant types of inheritance in cross variants in the group of "high cadmium" breeding samples and intergroup crosses. The total share of these types of phenotypic heredity was 85,7% in the crossing of "high cadmium" samples and 66,7% in intergroup crossings.

The created hybrids were evaluated in a field experiment with a natural background of cadmium. The average indicator of cadmium content in the seeds of hybrids for the experiment was 0,41 mg/kg with a range of variation from 0,05 to 2,24 mg/kg. The minimum values were noted in samples 19/06, 19/32 and 19/67 obtained in combinations of Rezon X PR63LLO1; Zorya X JG3 and JB231AC X X51B, respectively. The largest share of hybrids with a minimum cadmium content (≤ 0,1) was obtained in combinations using the line 56\3, commercial hybrids Rezon, Sumico as the maternal component and the commercial hybrid Sumico, lines X51B and JB231AC as the parent component (pollinator).

Based on the results of crossing, the range of variability and other selectively controlled traits was expanded, namely: the duration of the "seedling-flowering" period, the weight of 1000 seeds and the plant productivity indicator.

A detailed study of the physiological and transcriptomic mechanisms of cadmium accumulation was carried out on breeding samples 62\3 and JB231AC with high and low cadmium content, respectively. The studies were carried out at different concentrations of Cd, namely: 0, 25, 50, 100 μM. The results showed that JB231AC has a higher tolerance to Cd, compared to sample 62\3. Under cadmium stress, the content of H_2O_2 and MDA (malondialdehyde) in 62\3 was lower than that of JB231AC, but the activities of SOD (superoxide dismutase) and POD (peroxidase) in JB231AC were higher than those of 62\3, indicating that JB231AC had a higher ability to remove toxic substances caused by reactive oxygen species (ROS). It was found that ABC (ATP-binding cassette) and ZIP (Zn-regulated transporter, iron-regulated transporter-like protein) genes may play an important role in different levels of Cd accumulation in both cultivars. One NRAMP (*Natural resistance-associated macrophage protein*) gene was identified that was upregulated and had a higher expression level in 62\3.

In the applied aspect of the work (for conducting the next stages of selection), 5 hybrid samples were selected: 19/06; 19/32, 19/67, 19/40 and 19/41. The maximum level of cadmium content in the seeds of the samples was less than 0,1 mg/kg. The

range of indicators of plant productivity was 37,9-58,6 g/plant, productivity 2,1-3,2 t/ha, oil content in seeds 46,2-47,0%, which provides calculated indicators of oil yield at the level of 0,94-1,48 t/ha.

In addition, three samples (19/54, 19/11 and 19/26) were selected with typical features of cadmium-accumulating plant species. The high level of cadmium concentration in the above-ground part of plants (5 or more times higher than the natural background) allows us to consider the selected samples as the source material for creating of phytoremedial genotypes. The calculated level of removal of biologically active cadmium from the soil is at the level of 20,0 g/ha.

The created source material of sunflower undergoes further developing in the breeding programs of the Sumy National Agrarian University, the Institute of Agriculture of the Northeast of the National Academy of Sciences of the National Academy of Sciences, and the Henan Institute of Science and Technology.

A collection of intervarietal sunflower hybrids was transferred to the laboratory of selection and seed production of the Institute of Agriculture of the North East of the National Academy of Sciences, the results of research (which were published in specialized publications of Ukraine and international publications included in the Scopus database) are included in the curricula of the disciplines of the bachelor's degree program, specialty 201 - Agronomy.

Key words: *sunflower, selection, breeding, collection, samples, hybryds, inheritance, indicator, inheritance, Cd accumulation, hybridization, resistance, physiology mechanism, transcriptomic, correlation, self-pollinated lines, productivity, seed quality, yieald.*

АННОТАЦІЯ

Фу Юаньчжі. Селекція соняшнику на стійкість до накопичення кадмію. Рукопис дисертації на здобуття наукового ступеня доктора філософії (PhD) за спеціальністю 201 «Агрономія». – Сумський національний аграрний університет, Суми, 20220

Соняшник є основною олійною культурою в Україні, та має домінуючий вплив на формування світового ринку харчових олій. Відповідно до стандартів ЄС вміст кадмію в олії соняшнику не повинен перевищувати 0,05 мг/кг. Одним із шляхів забезпечення нормативних показників в умовах зростання рівня забруднення кадмієм є використання сортів соняшнику з низьким рівнем накопичення цього металу. Використання таких сортів може ефективно зменшити вміст кадмію в насінні, принципово вирішуючи проблему його надходження в організм людини через продукти переробки урожаю соняшнику.

У процесі досліджень проведено скринінг селекційних зразків соняшнику за ознакою стійкості до накопичення кадмію. Вивчено фізіологічні та транскриптомні механізми накопичення Cd в окремих частинах рослин соняшнику. Як тестовий матеріал, в експерименті було задіяно 104 селекційні зразки соняшнику. Біомаса рослин та вміст кадмію використовувалися як скринінгові показники у горщиковій культурі, гідропоніці та польових умовах. Було відібрано сорти зі значною різницею в накопиченні кадмію та отримано експериментальні матеріали для вивчення механізмів накопичення кадмію в різних частинах рослин.

За результатами вегетаційного досліду з використанням аналізуючого фону з концентрацією кадмію 1,0 мг/кг було встановлено, що діапазон значень показника концентрації цього металу в надземній частині рослин коливався від 0,5 до 2,6 мг. /кг. В межах цього діапазону було виділено п»ять груп із різним рівнем стікості до накопичення кадмію

Високу стійкість до накопичення кадмію в надземній частині рослин (0,5-0,9 мг/кг) відзначено у 11 зразків: JB231AC, S2K670, UE0100938, IPS\125, 64\3, Polaris CL, Reason, 56\3, Snk630, UE0100977 та UE0100018. Частка цієї групи в

колекції становила 10,6%. До групи з дуже низьким рівнем стійкості (вміст кадмію більше 2,11 мг/кг) увійшли 7 селекційних зразків PR63LL01, UE0100056, UE0100715, 62\3, UE0100052, JG3, UE0100060. Частка цієї групи в колекції була 6,7%.

Наступним етапом роботи було визначення стійкості рослин до накопичення кадмію у насінні (95 зразків). Дослідження проводилися в умовах польового досліду з природним фоном кадмію в грунті 0,21 мг/кг. За результатами 3-річного експерименту діапазон показника вмісту кадмію коливався від 0,23 до 0,43 мг/кг. Середнє значення цього показника в насінні для колекції становило 0,34±0,01 мг/кг. Значення коефіцієнта варіації склало 12,12%.

Порівняння даних вегетаційного та польового дослідів показало подібність результатів щодо диференціації колекції за рівнем стійкості до накопичення кадмію. Більше половини, а саме 63,8% селекційних зразків, проаналізованих методом непрямої оцінки, відповідали показникам, отриманим у польовому досліді. Найвища частка спільних (для обох дослідів) зразків була відмічена у групах із низьким та незадовільним рівнями стійкості. Високою часткою спільних гібридів, а саме 76,1 %., також, характеризувалася група з високим рівнем стійкості до накопичення кадмію.

Часта спільних гібридів у групах з добрим та задовільним рівнями стійкості складала 40,6 та 42,8% відповідно. У випадках «групової невідповідності» спостерігалося зміщення селекційного зразка до наступної (вищої або нижчої) групи. Отже, використання методу непрямої оцінки забезпечує високу ефективність негативного добору (проти ознаки). Цей метод може бути використаний для виключення зразків із низьким рівнем стійкості до накопичення кадмію із селекційної програми.

Детальний аналіз результатів диференціації колекції різними методами досліджень вказує, що висока частка спільних (для обох дослідів) зразків у групах із низькою та незадовільною стійкістю може бути результатом універсальності генетичних механізмів високого рівня накопичення кадмію у вегетативних органах і насінні соняшнику.

За результатами вивчення базової колекції в умовах польового досліду визначено середні значення та діапазон варіювання основних селекційно контрольованих ознак. Середнє значення показника тривалості періоду «сходи-цвітіння» становило 56,5 дня з діапазоном мінливості від 50 до 66 днів. Коефіцієнт варіації склав 6,81%. Середнє значення, діапазон мінливості та коефіцієнт варіації базової колекції за продуктивністю рослин становили відповідно 33,6 г, 11,70–63,30 г та 20,75 %.

За результатами кластерного аналізу встановлено морфологічну неоднорідність у групі низькокадмієвих зразків із виділенням трьох внутрішньогрупових кластерів. Перший кластер включав пізньостиглі (тривалість фази «сходи-цвітіння» > 64 днів), високорослі (> 193 см) та низьковрожайні зразки UE0100114, UE01000114, UE0100977, UE0100018. Другий кластер охоплював зразки із середнім і вищим рівнем продуктивності рослин (22,0–52,0 г/рослину). Окремий кластер утворили зразки зі специфічними характеристиками. Гетерогенність групи низькокадмієвих зразків передбачає наявність різних механізмів формування насіннєвої продуктивності та реалізації ознаки стійкості до накопичення кадмію. Навпаки, однорідність групи з «високим рівнем накопиченням кадмію» може вказувати на наявність єдиного механізму транспортування біологічно активного кадмію від вегетативних органів до насіння.

Неоднорідність базової колекції селекційних зразків та відмінність у механізмах забезпечення низького рівня накопичення кадмію в насінні соняшнику підтверджено результатами міжсортових схрещувань. Отримано та досліджено 73 зразки міжсортових гібридів у F_1 та F_2. Встановлено такий результат: при схрещуванні «малокадмієвих» батьківських форм лише п’ята частина або 19% з них успадкувала ознаку стійкості до накопичення кадмію за типом гетерозису. Частки гібридів, які успадкували ознаку за типом часткового позитивного домінування та проміжним типом для батьківських форм, становили 9,5 % на кожну. У більшості випадків 62% успадкування ознаки відбувалося за типом депресії та часткового негативного домінування. Депресія та часткове негативне домінування також були переважаючими типами успадкування у варіантах схрещування у групі «висококадмієвих» селекційних зразків та міжгрупового схрещування. Сумарна частка цих типів фенотипової спадковості становила 85,7% при схрещуванні зразків «з високим вмістом кадмію» і 66,7% при міжгрупових схрещуваннях.

Було проведено оцінювання створених гібридів у польовому досліді з природним фоном кадмію. Середній для досліду показник вмісту кадмію у насінні гібридів склав 0,41 мг/кг із діапазоном варіювання від 0,05 до 2,24 мг/кг. Мінімальні значення було відмічено у зразків 19/06, 19/32 та 19/67, отриманих в комбінаціях Rezon X PR63LLO1; Зоря X JG3 та JB231AC X X51Б, відповідно. Найбільшу частку гібридів із мінімальним вмістом кадмію (≤ 0,1) було отримано у комбінаціях із використанням як материнського компонента лінії 56\3, комерційних гібридів Rezon, Sumico та як батьківського компонента (запилювача) – комерційного гібрида Sumico, ліній X51Б та JB231AC.

За результатами схрещування розширено діапазон мінливості і інших селекційно контрольованих ознак, а саме: тривалості періоду «сходи-цвітіння», маси 1000 насіння та показника продуктивності рослин.

Детальне вивчення фізіологічних і транскриптомних механізмів накопичення кадмію було проведено на селекційних зразках 62\3 та JB231AC із високим та низьким вмістом кадмію відповідно. Дослідження проводилися при різних концентраціях Cd, а саме: 0, 25, 50, 100 мкМ. Результати показали, що JB231AC має більш високу толерантність до Cd, порівняно із зразком 62\3.

Під впливом «кадмієвого стресу» вміст H_2O_2 і MDA (малоновий діальдегід) у 62\3 був нижчим, ніж у JB231AC, однак активність SOD (супероксиддисмутази) і POD (пероксидази) у JB231AC були вищими, ніж у 62\3, що вказувало на те, що JB231AC мав вищу здатність видаляти токсичні речовини, спричинені активними формами кисню (АФК).

Було встановлено, шо гени ABC (АТФ-зв'язувальні транспортні білки) і ZIP (Zn-регульований транспортер, залізо-регульований транспортер-подібний білок) можуть відігравати важливу роль у різних рівнях накопичення Cd у обох сортів. Було ідентифіковано один ген NRAMP (природний білок макрофагів, пов’язаний із резистентністю), який має підвищену регуляцію та вищий рівень експресії в 62\3.

У прикладному аспекті роботи (для ведення наступних етапів селекції) було відібрано 5 гібридних зразків: 19/06; 19/32, 19/67, 19/40 та 19/41. Максимальний рівень вмісту кадмію в насінні зразків був менший 0,1 мг/кг. Діапазон показників продуктивності рослин склав 37,9-58,6 г/рослину, урожайності 2,1-3,2 т/га, вмісту олії у насінні 46,2-47,0 %, що забезпечує розрахункові показники виходу олії на рівні 0,94-1,48 т/га.

Крім того було виділено три зразки (19/54, 19/11 та 19/26) з ознаками, характерними для видів рослин-накопичувачів кадмію. Високий рівень концентрації кадмію у надземній частині рослин (у 5 і більше раз перевищує показники природного фону) дозволяють розглядати виділені зразки як вихідний матеріал для створення генотипів фіто меліоративного напряму. Розрахунковий рівень винесення біологічно активного кадмію із грунту є на рівні 20,0 г/га

Створений вихідний матеріал соняшнику проходить подальшу доробку у селекційних програмах Сумського НАУ, Інституту сільського господарства Північного Сходу НААН та Хенанського науково-технологічного інституту.

До лабораторії селекції та насінництва Інституту сільського господарства Північного Сходу НААН передано колекцію міжсортових гібридів соняшнику, результати досліджень (які були опубліковані у фахових виданнях України та міжнародних виданнях включених до БД Scopus) включено до навчальних програм дисциплін ОС бакалавр, спеціальності 201 – Агрономія у Сумському НАУ.

Ключові слова: *соняшник, селекція, добір, колекція, зразки, сорти, гібриди, успадкування, показник успадкування, накопичення Cd, гібридизація, стійкість, фізіологічний механізм, транскриптомія, кореляція, самозапилені лінії, продуктивність, якість насіння, урожайність.*

LIST OF PUBLISHED WORKS ON THE TOPIC OF THE DISSERTATION

Articles in scientific journals with an impact factor (Scopus, WS)

1. **Fu Yuanzhi**, Zhatova Halyna, Li Yuqing, Liu Qiao, Trotsenko Volodymyr, Li Chengqi. Physiological and transcriptomic comparison of two sunflower *(Helianthus annuus* L.) cultivars with high/low cadmium accumulation [J]. Frontiers in Plant Science, 2022, 13. DOI: 10.3389/FPLS.2022.854386

Articles in professional publications of Ukraine

2. **Fu Yuanzhi**, Wu Liuliu, Trotsenko V., Zhatova H. Screening of variety collections of sunflower and winter wheat for cadmium low accumulation.- Bulletin of Sumy National Agrarian University. The series “Agronomy and Biology, 3 (37), 2019, 42-47.

3. **Fu Yuanzhi,** Trotsenko Volodymyr. Accumulation of heavy metals in sunflower seedlings under the influence of cadmium stress. Bulletin of Sumy National Agrarian University. The series “Agronomy and Biology, 3 (45), 2021, 64-70. DOI https://doi.org/10.32845/agrobio.2021.3.8

4. **Fu Yuanzhi,** Trotsenko Volodymyr. Ways of the cadmium accumulation monitoring in sunflower and other crops: overview. - Bulletin of Sumy National Agrarian University. The series “Agronomy and Biology, 4 (46), 2021, 89-96. DOI https://doi.org/10.32845/agrobio.2021.4.13

Abstracts of conferences

5. Trotsenko V.I., Rogulskyi Yu.V., **Fu Yuanzhi.** Effectiveness of the method of indirect assessment of sunflower breeding material for resistance to cadmium accumulation. - World plant resources: state and prospects of development: Materials of the V International Science. - practice conference (June 7, 2019, Kyiv) / Ukrainian Institute of Plant Varieties Expertise. Vinnytsia: Nilan-LTD, 2019., 60.

6. **Yuanzhi Fu**, Trotsenko V. Breeding of sunflower with low cadmium content. - Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya», (24-25.05.2019) , 2019, 83.

7. Trotsenko V. I., Zhatova G. O., **Fu Yuanzhi**. Analysis background parameters for testing sunflower for resistance to cadmium accumulation. - Oil crops: innovations and prospects. Collection of theses of the International Scientific Internet Conference (May 14, 2019). Zaporizhzhia. IOK NAAS, 2019, 45-46.

8. **Fu Yuanzhi**. The current state of sunflower crop in China. - Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya», (25-26.05.2020), 2020, 103.

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10. **Fu Y.**, Trotsenko V., Zhatova H. Low cd breeding varieties in sunflower. Trends and prospects development of science and practice in modern environment X International Scientific and Practical Conference Geneva, Switzerland (November 22 – 24, 2021), 15

11. Trotsenko V., **Fu Yuanzhi**. Status and prospects of Cadmium control in sunflower seeds - Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya», (25.05.22), 2022, 209-211.

LIST OF CONVENTIONAL ABBREVIATIONS

Ascorbic acid (AsA)

ATP-binding cassette (ABC)

Boron (B)

Catalase (CAT)

Copper (Cu)

Differential expression of genes (DEGs)

False discovery rate (FDR)

Fold change (FC)

Glutathione (GSH)

Glutathione reductase (GR)

Heavy metal associated isoprenylated plant protein (HIPP)

Heavy metal ATPase (HMA)

Iron (Fe)

Lithium (Li)

Manganese (Mn)

Metal tolerance protein (MTP)

Metal-nicotianamine transporter (YSL)

Molybdenum (Mo)

Natural resistance-associated macrophage protein (NRAMP)

Nickel (Ni)

Peroxidase (POD)

Reactive oxygen species (ROS)

Selenium (Se)

Strontium (Sr)

Superoxide dismutase (SOD)

Transfer factor (TF)

Trace metallic elements (TMEs)

Zinc (Zn)

Zn-regulated transporter, Iron-regulated transporter-like protein (ZIP)

CONTENT

INTRODUCTION

Sunflower is a relatively young crop. V. Pustovoit, P. Leclercq, M. Kinman, and V. Kiritchenko played a decisive role in the formation of the theoretical basis and practical directions of sunflower breeding. Currently, sunflower is grown on an area of about 30 million hectares, providing up to 10% of the world oil market. Crop feature is the ability to accumulate in the seeds of dangerous heavy metal Cd. In the world, the problem of cadmium is determined by the steady increase in the concentration of this element in the lands of agricultural use primarily as a result of the of mineral fertilizer applicaion. It will gradually accumulate and harm crops for a long time through the transmission of the food chain leading to the decline of crop yield capacity and quality. Cadmium can accumulate in the human body through the food chain and induce many diseases affecting human health. Therefore, how to achieve reducing Cd accumulation in grains has become an important research topic worldwide.

Facing the increasingly severe problem of improving the soil polluted by cadmium, cultivation of sunflower varieties with low cadmium accumulation can effectively reduce the cadmium content in seeds, and fundamentally solve the problem of cadmium entering the body through the sunflower crop.

By formulating hybridization and backcross cultivation strategy, evaluation and selection of hybrid offspring materials and new varieties (lines) with low Cd, high quality, and good comprehensive characteristics have some achievements. In addition, physiologically, the characteristics of uptake, transport and distribution of Cd in different organs are still an important basic research topic for low Cd breeding. The mechanisms of molecular and metabolic regulation of signal transduction in crops under Cd stress are still unknown and need to be clarified.

Therefore, it is necessary to establish an efficient and rapid selection system in sunflower by systematically analyzing the molecular basis of agronomic traits associated with Cd accumulation, exploring the available excellent gene resources, innovating and integrating classics breeding and molecular design breeding.

Actuality of theme. The prospect of a significant increase in the cadmium concentration in the near future as well as the growing demands on the quality of plant products need the search for methods of breeding control of this trait in sunflower. Currently, the implementation of this direction is constrained by the lack of effective, theoretically found methods of creating specialized genotypes with a controlled level of cadmium accumulation.

Connection of work with scientific programs, plans, topics. 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); Scientific topic of the Institute of Agriculture of the Northeast of the NAAS of Ukraine "Development of a genotype model and creation of starting material of sunflower resistant to cadmium accumulation" (state registration number 0121U108674, 2021-2023).

Object of study. Regularities of manifestation of morphological and genetic traits in sunflower collection samples and establishment of breeding value on their basis.

Subject of study. Features of manifestation of a resistance traits to cadmium accumulation in sunflower.

Research methods. general scientific - analysis, induction, deduction, synthesis and generalization; field - phenological observations of the sunflower collection to determine the selection value of samples; special breeding - determination of biometric and allometric parameters of growth and development of plants, in particular measuring and weight, structural analysis; statistical - variance, variation, correlation, cluster analysis to determine the patterns of variability of quantitative and qualitative characteristics.

Scientific novelty of the obtained results. The scientific novelty of the obtained results lies in solving an important scientific problem of creating and evaluating the source material of sunflower with a low level of cadmium accumulation.

For the first time: a method of indirect evaluation of selected sunflower samples for resistance to cadmium accumulation was developed and tested. The methodology is based on conducting a vegetation experiment with an analyzing background of cadmium concentration of 1,0 mg/kg, (cadmium source – cadmium monosulfate), plant vegetation up to the R5 phase;

A working collection was formed with a range of cadmium content in the above-ground part of plants (background 1,0 mg/kg) – 0,5-2,6 mg/kg in seeds (background 0,21 mg/kg) – 0,23-0,43 mg/kg. Samples with a high level of differentiation based on resistance to cadmium accumulation were selected;

It was established that the predominant type of inheritance of the trait of resistance to cadmium accumulation is depression and partial negative dominance. The total value of these types was: when crossing low cadmium genotypes - 62%, when crossing high cadmium genotypes and between group crossing – 85,7 and 66,7% respectively;

The results showed that JB231AC had better Cd tolerance than 62\3. The contents of H_2O_2 and MDA (malondialdehyde) in 62\3 were lower than that in JB231AC under Cd stress, but the activities of SOD (superoxide dismutase) and POD (peroxidase) in JB231AC were higher than in 62\3, which indicated that JB231AC had a strong ability to remove reactive oxygen species (ROS)-induced toxic substances.

Many deferentially expressed *ABC* (*ATP-binding cassette*) and *ZIP* (*Zn-regulated transporter, Iron-regulated transporter-like protein*) genes indicated that the two gene families might play important roles in different levels of Cd accumulation in the two cultivars. One up-regulated *NRAMP* (*Natural resistance-associated macrophage protein*) gene was identified and had a higher expression level in 62\3.

The original source material of sunflower was created, with a controlled level of cadmium accumulation and the estimated productivity of oil type genotypes – 0,94-1,48 t/ha t oil/ha. For the genotypes of the phytoremediation direction, the calculated rates of removal of biologically active cadmium from the soil were 20,0 g/ha per growing season.

The scheme for evaluating collection samples of cross-pollinated plant species by yield quality indicators has been improved.

The issue of increasing the level of adaptability to environmental conditions while narrowing the genetic base of the species population has gained further development.

Practical significance of the obtained results. According to the results of the research, the collection of sunflower with a low level of cadmium accumulation was transferred to the Institute of Agriculture of North-East of Ukraine; materials of articles and annual reports are used in the in educational programs on disciplines of educational level of Bachelor of 201 Agronomy specialty at Sumy NAU.

Applicant's personal contribution. It was planned and carried out of research, it was summarized the data of the scientific literature on the topic of the dissertation, it was analyzed of experimental data, it was formulated the conclusions and recommendations for breeding, it was prepared and written of scientific papers. Scientific articles have been published both independently and in co-authorship.

Approbation of dissertation results. The results of the research were published and discussed at 7 conferences. The main items, research results and conclusions of the work during 2019-2022 were presented and discussed at the meetings of the Department of Plant Production of Sumy National Agrarian University.

Publications. The main items of the dissertation are covered in 11 publications, including 3 articles in professional publications of Ukraine, one in an international journals included in the database of Scopus, 7 abstracts of International Conferences.

The structure and content of the dissertation. The dissertation contains an annotation, an introduction, four sections, conclusions, a list of references and appendices. The dissertation is presented on 195 pages of computer text, in particular 137 pages of the main text. The work is illustrated with 19 tables, 28 figures and 10 appendices.

CHAPTER 1

REVIEW OF LITERATURE AND CHOICE OF RESEARH DIRECTION

1.1 Sources of cadmium pollution and its risks

Cadmium pollution in soil. Cadmium (Cd) is a ductile gray heavy metal, located in group IIB of the periodic table, with atomic number ‘48’ and a molecular weight of 112,4 (Public, 1992). For the first time, Cd was discovered by Stromeyer in 1811 while purifying zinc oxide compounds. In the natural environment, Cd rarely exists in its elemental form. It is available in the soil solution primarily as Cd^{2+}, as well as Cd-chelates. The content of Cd in the earth’s crust is very low. Throughout the world, the content of Cd in soil ranges from 0,01 to 2 mg/kg, with an average of 0,35 mg/kg (Yang et al., 2010), Due to its strong mobility and high phytoavailability in soil, Cd is easily taken up by plant roots (Huang et al., 2021; Sahito et al., 2022; Shahid et al., 2017; Yu et al., 2020).

Cd occurs naturally in rock and is generally found in low concentrations in soil. Cd contaminated soil is mainly the result of human activities, which can be summarized into three aspects:

1) Atmospheric deposition, industrial exhaust, and automobile exhaust are the main sources of atmospheric Cd pollution. Cd in the atmosphere can enter the soil environment through dust, rain, and other sedimentation and become one of the main sources of soil Cd pollution (Murtaza et al., 2015; Yan et al., 2021a). Enrichment of rice with different sources of exogenous Cd supply through pot simulation experiments was studied and it has been found that the degree of Cd uptake into the plant from different pollution sources was in the order of atmospheric deposition: pollution > irrigation water pollution > simulated soil pollution. (Zhou et al., 2020b)

2) Sewage irrigation and sludge utilization. With the rapid development of mining, metallurgy, electroplating, and battery industry, a large amount of wastewater containing Cd is discharged into farmland as irrigation water without treatment or the treatment can’t meet the national regulations (Ali et al., 2021; Liñero et al., 2018; Monu et al., 2008; Ramlan et al., 2021).

3) The application of pesticides and chemical fertilizers. Production of pesticides and fertilizers is often accompanied by Cd (Grant et al., 2013; Murtaza et al., 2015; Zhu et al., 2020). Cd levels in topsoil in some areas of northern France are as high as 300 mg/kg (Sterckeman et al., 2000).

Cd content of 280 mg/kg was also found in contaminated paddy soils in Thailand (Simmons et al., 2005). The total land acreage where the soil is above the safety threshold level was 16,1% in China, with Cd, nickel, and arsenic being the top three inorganic pollutants, with frequencies of soils above the threshold concentrations being 7,0%, 4,8%, and 2,7%, respectively. Among them, Cd pollution is the most serious. Furthermore, Cd pollution in the soil arable layer in China is increasing at an average rate of 0,004 mg/kg per year, which is much higher than its rate in Europe (Luo et al., 2009; Fu & Trotsenko, 2021).

Cd toxicity. Cd is a toxic heavy metal, and excessive Cd in plants will affect the normal physiological functions of plants (Cornu et al., 2020; Dias et al., 2013; He et al., 2017; Jaouani et al., 2018; Lv et al., 2019a; Rabêlo et al., 2021).

Cd poisons plants in two ways: 1) a large amount of free Cd^{2+} accumulates in plant cells, which interferes with the original ion balance and redox potential of the cells, resulting in the obstruction of ion absorption and transport, the imbalance of cell osmotic pressure, and damaging normal metabolic process. 2) Cd combines with macromolecular substances such as nucleic acids, proteins, enzymes, etc., or replaces the central ions of these macromolecules denaturalizing and inactivating them.

Its incorporation in plants has been reported to induce severe phytotoxic effects as it restricts the bio-synthesis of chlorophyll (Shahabivand et al., 2017), alters water status (Barcelo & Poschenrieder, 1990), reduces growth, particularly roots, interrupts with mineral uptake and carbohydrate metabolism (Wang et al., 2008), encourages stomatal closure (Zhu et al., 2020), retards the photosynthetic mechanism (Rabêlo et al., 2021), impairs the process of transpiration (Liñero et al., 2016), respiration and nitrogen assimilation (Wang et al., 2008), and consequently lowers biomass production (Ahmad et al., 2015; Barcelo & Poschenrieder, 1990; Fan et al., 2011; Jaouani et al., 2018; Qian et al., 2009; Wang et al., 2008; Zhou & Qiu, 2005). Most of the Cd absorbed by the human body comes from the enrichment of the food chain and selectively accumulates in the kidneys and liver (Qi et al., 2020; Yang et al., 2010).

Kidneys accumulate up to 1/3 of the total amount and are the target organ of Cd poisoning. Excessive Cd enrichment can cause renal function decline (such as renal tubular cell proliferation, necrosis, or atrophy, etc.) and metabolism obstruction (such as glycosuria, proteinuria, and amino acid urine) (Reyes-Hinojosa et al., 2019; Templeton & Liu, 2010).

Among the Cd poisoning incidents, the most influential one was the “Itai-Itai disease” in the 1960s. Residents had been consuming water and rice polluted by Cd. Thus, cadmium entered the human body. It was not decomposable and accumulated for a long time, which led to joint pain, bone deformity, easily broken bones, and finally death (Aoshima, 2012).

Since the toitaka disease incident in Fukuyama prefecture of Japan was identified as the result of soil Cd pollution in the 1960s (Qi et al., 2020), cases of human Cd poisoning caused by Cd pollution have been reported in other parts of the world (Bakulski et al., 2020; Genchi et al., 2020; Reyes-Hinojosa et al., 2019).

Therefore, many countries in the world have formulated limit standards for heavy metals in some fertilizers. How to prevent or mitigate heavy metal soil pollution of cropland and ensure safe food production has become an important issue in the modern world.

1.2 Cd accumulation, uptake, and its transportation by plants

Uptake and transportation of Cd. Cd is mainly taken up into the plant through the root system. Available Cd in the soil is absorbed into the root system passively or actively through the symplast pathway, then transported to the aboveground tissues through the xylem, powered by transpiration, and accumulated into the grains through the internode phloem (Uraguchi & Fujiwara, 2012; Uraguchi et al., 2009).

Cd transportation in plants is mediated by transporters of essential elements, such as zinc, calcium, iron, and manganese with Cd uptake and xylem loading in the roots and remobilization from leaf blades and intervascular transfer in the nodes to redirect Cd transportation, which is crucial for the grain Cd accumulation (Shimpei & Toru, 2013). The root cell wall is the first barrier to prevent Cd from entering the plant body, and the Cd uptake rate is proportional to the Cd concentration in the hydroponic culture medium in the range of 0,05–100 nM (Fujimaki et al., 2010).

Sunflower growth was not limited when exposed to 20 nM Cd. The amount of Cd taken up by the plant roots, as well as the rate of Cd loading in xylem sap, increased in direct proportion to the concentration of Cd^{2+} in the nutrient solution (Cornu et al., 2016). The effects of cerium oxide nanoparticles and Cd on maize seedlings were observed and measured, analyzing the changes in root anatomical structure. It was found that there was a clear apoplastic barrier near the root tip, which was the physiological response of the root as the resistance to heavy metal uptake (Fox et al., 2020).

After Cd enters the root cells, its part is sequestered in vacuoles in the form of a Cd-plant chelate protein complex (Miyadate et al., 2011; Ueno et al., 2010), while the rest is transported to the xylem. The separation of Cd into vacuoles is considered an effective tolerance mechanism, which reduces Cd transport to the grains (Gao et al., 2016; Sadiq et al., 2017; Xin et al., 2018).

Plants can also minimize the concentration of free Cd in the cytosol by forming metal chelates or complexes with phytochelatins or metallothioneins (Saraswat & Rai, 2011).

Hart et al. studied the biological processes in durum wheat of root Cd uptake, xylem Cd translocation to shoots, and Cd accumulation in wheat grains. Excessive Cd accumulation in durum wheat grains was not correlated with either seedling root influx rates or root-to-shoot translocation but might be related to the phloem-mediated transportation of Cd to the grains (Hart et al., 1998).

It was shown that Cd accumulation in rice grains was independent of root uptake time and Cd concentration in soil but was strongly positively correlated with the Cd concentration in the xylem with the Cd translocation *via* xylem from root to shoot being the major physiological process to determine the Cd concentration accumulated in rice grains (Shimpei et al., 2009). The contribution of rice phloem to the transfer of Cd to grains was studied and it showed that 91–100% of Cd in grains was deposited in the phloem. During reproductive growth, Cd is absorbed from the roots and transported to the grains *via* stems and leaves (Kensuke et al., 2007).

It is deposited in the developing grains (Harris & Taylor, 2013) with the nodes being the central organ where xylem-to-phloem transfer takes place, and which play a key role in the process where Cd is transferred from the soil to the grains at the grain-filling stage.

The spatial distribution and dynamic changes of Cd in wheat and low-Cd wheat during their development process were studied (Shi et al., 2019). The results showed that the Cd concentrations in grains of the two varieties were significantly different, but those in the rachis and the glumes were similar between the two varieties, which indicated that the two varieties had different regulatory mechanisms regarding the retransfer and redistribution of Cd from the rachis and the glumes to the grains during the reproductive stage.

Cd accumulation in crops. At present, the Cd concentration in grains in most areas in the world is within the safe range, although these values show regional differences (Shakerian et al., 2012). The food safety standards operated by the European Union and China stipulate that the Cd limit in rice is 0,2 mg/kg, and the Cd limit of the Codex Alimentations Commission (CAC) is 0,4 mg/kg (Yang et al., 2019).

According to EU standards, sunflower Cd accumulation should not be more than 0,05 mg/kg. However, Cd can be accumulated to a relatively high level with no disadvantage to its ontogenesis (Grant et al., 2008). Two hundred sunflower genotypes were evaluated at four different soil series in North Dakota and Minnesota. Large genetic variation in Cd content was found among genotypes. Kernel Cd concentrations showed continuous variation across the range of 0,31 to 1,34 mg/kg (average for four locations) (Li et al., 1995).

The latest research reported that, worldwide, grain Cd averaged 0,093 mg/kg, although mean values varied 16-fold between regions, with South China (0,32 mg/kg) > Argentina (0,15 mg/kg) = German (0,13 mg/kg) > Japan (0,11 mg/kg) > the

United States (0,064 mg/kg) > Central-North China (0,020–0,60 mg/kg) ≥ Iran (0,042 mg/kg) >Brazil (0,023 mg/kg) = South Korea (0,020 mg/kg) (Zhang et al., 2021b).

The study showed a range of Cd concentrations in grains (from below the detection limit (BDL) to 0,49 mg/kg) and soil samples (1,76 mg/kg to 13,8 mg/kg) collected from agricultural fields located at different regions in West Bengal, much higher than the allowable limits (Majumdar et al., 2020).

In Egypt, the levels of Cd in soil and maize grains irrigated by low-quality water contaminated possessed multiple pollutants in comparison with those irrigated by freshwater. Cd levels in maize kernels harvested from crops irrigated with low-quality water were almost within the permissible limits (0,1 mg/kg) and were 30-times higher than those in kernels from maize crops irrigated with freshwater (El-Hassanin et al., 2020).

In Germany, Cd uptake study of 602 soybean accessions showed an average Cd content of 0,13 mg/kg, with about 12,5% of the materials exceeding the EU limit (Franzaring et al., 2019). In New Zealand, Cd content in grains of 12 wheat varieties from different regions was investigated, with Cd concentration in the grains ranging from 0,004 to 0,205 mg/kg, with an average of 0,066 mg/kg, and 7% of the varieties exceeding 0,1 mg/kg (Gray et al., 2019).

According to incomplete statistics, Cd concentration in grains in most regions of most countries is below the limit, although it is easy to exceed the limit in a few regions or areas dominated by mining or heavy industry.

In addition, different crops or varieties have different Cd accumulation abilities in grains. A study showed that *indica* was easier to accumulate Cd than *japonica*, and the Cd content in grains of *indica* rice was 1,84-4,14 times higher than *japonica* (Li et al., 2019a).

The Cd content of *indica* varieties may be higher than the national Cd limit under medium and low Cd-contaminated soil conditions (Chen et al., 2019). Pan et al. studied the Cd accumulation in the grains of 30 wheat varieties when the soil pollution of Cd was 0,38mg/kg, the Cd concentrations in the grains of 29 varieties were below the limit values (0,1 mg/kg), and all of them were lower than limit values of 0,2 mg/kg of the CAC and European Union. (Pan et al., 2020a).

Cd distribution in plants. Cd accumulation capacity is different in different organs of the same plant (Liñero et al., 2018). According to the absorption and transportation characteristics of Cd, most of the research results revealed that the order of Cd distribution in organs was basically as follows: root > stem > leaf > grain (He et al., 2017; Jun et al., 2020; Maria et al., 2014; Zhou & Qiu, 2005; Chen et al., 2014; Liu et al., 2014; Zhao et al., 2006).

The characteristics of Cd accumulation in vegetative organs of 10 *indica* varieties were studied. The results showed that the content of Cd in roots was the highest – 4-13 times that of leaves, 8-10 times that of cob, and 20-40 times that of grains.

During grain-filling, a large amount of Cd in leaves was exported to grains, so there was a high correlation between leaves and grains with a correlation coefficient of 0,769 (Wen et al., 2006).

For different rice varieties, the difference in Cd accumulation in grains mainly occurs at the reproductive stage (Kun et al., 2019) combined with the measures reducing Cd at the early stage of crop growth.

Application of appropriate agronomic measures, which reduce the Cd availability in the soil and control the Cd uptake and transportation to the grains can effectively reduce the Cd content in grains at the grain-filling stage (Yu et al., 2018).

Yan et al. studied the allocation of Cd in wheat organs by isotopic tracer during the flowering and grain maturation stage. He found that when the Cd concentration was high, the proportion of Cd transferred from the root to stem and aboveground, Cd distribution to grains was also high and Cd migrating at the flowering stage accounted for 40-45% of Cd accumulation in grains on average.

Cha et al. found that the order of Cd distribution concentration in all structures of rice or wheat grains was generally the same. The order was as follows: cortex > embryo > endosperm > glume. The order in maize – embryo > endosperm > cortex. The total Cd content in endosperm had an absolute predominance in various structures of the grain accounting for 64-70% (Cha et al., 2000)

Sunflowers were subject to six levels of soil contamination (from 2,5 to 15 mg/kg Cd) with the untreated control, from the emergence of the cotyledon leaves until the harvest when sunflowers were at the flower bud stage. An overall increase of Cd concentration was found in all tissues of the plants (roots, stem, young, mature, and old leaves) with increasing Cd contamination in the soil. Regardless of treatments, Cd concentration in roots always exceeded those in the aboveground dry matter with a low translocation from roots to shoots (Alaboudi et al., 2018; Hawrylak-Nowak et al., 2015; Maria et al., 2013; Sadiq et al., 2019).

Regarding Cd accumulation in sunflower seeds, the results indicated that Cd is translocated to seeds, and the cotyledons showed the highest concentration (Cd-high group) ranging from 10 to 20μg g^{-1}.

Considering both total concentration and the distribution in the seeds, Cd uptake causes the homeostasis misbalance of micronutrients (Pessôa et al., 2017).

When sunflowers were grown hydroponically in the greenhouse being exposed to low concentrations of Cd ($pCd^{2+} = 11{,}03$), there were no significant effects on the partitioning of recent Cd. Most of the recent Cd was recovered in roots (60%) and only 2,8% were found in seeds (0.8% for the husk and 2.0% for the kernel) (Liñero et al., 2016).

Physical and chemical factors affecting Cd uptake. Cd uptake in most plants increases rapidly with the increase of Cd concentration in soil, and the accumulation level of Cd in plants is significantly correlated with the content of the total available Cd in soil. There are many forms of Cd in soil, including exchange state, carbonate bound state, iron manganese oxide bound state, organic matter bound state, and residue state. The exchangeable Cd is the most active in soil and is called the available Cd which can be absorbed by plants together with the water-soluble Cd. The conversion between available and ineffective Cd states determines the availability of Cd in soil. Thus, soil physical and chemical factors will affect the level of Cd available in soil affecting the uptake of Cd in a plant.

The influence of soil physical and chemical factors on the level of available Cd is as follows:

① Soil PH. The solubility of Cd in the soil is negatively correlated with the soil PH value. The decrease of soil PH causes the absorption of Cd^{2+} in the soil to be resolved into the soil, increasing available Cd;

② Soil Eh (Oxidation-reduction potential). Under high Eh, the invalid Cd in the soil is more easily converted to water-soluble and exchangeable Cd. The exchangeable Cd content has a significant positive correlation with the soil Eh value;

③ Soil ions. Fertilizers containing Ca^{2+}, Mg^{2+}, Zn^{2+}, K^{+}, and other cations can compete with Cd^{2+} to reduce the adsorption of Cd in soil and compete with Cd^{2+} to enter plants to reduce the absorption of Cd in plants to a certain extent;

④ The content of soil organic matter. Cd^{2+} can be complexed with organic matter in the soil. Thus, ion exchange and adsorption can occur, which reduce Cd mobility and availability as they reduce available Cd in soil.

1.3 Physiological effects of Cd accumulation in plants

Regulation of crop growth under Cd stress. Many studies have shown that Cd distribution in rice plants is related to the physiological mechanism for Cd tolerance (Chiao et al., 2019a). Growth changes are often the most obvious response of plants to Cd stress (Chen et al., 2017b), which can cover the whole growth period from seed germination to crop maturity (Hakla et al., 2021). Crop growth is inhibited as the breakdown of the metabolic system, the weakening of photosynthesis and respiration, the imbalance of plant hormones are caused by Cd toxicity (He et al., 2017; Lin et al., 2018).

Firstly, Cd poses significant problems for successful seed germination and establishment. Fresh and dry masses of crop seedlings decreased significantly under 80 μM Cd stress (Chen et al., 2017b). Cotyledons and the emergence of young leaves of mung bean were reduced by 13% and 74%, respectively, under Cd stress, compared with control plants; root length and root surface area were significantly reduced, and plant height and stem diameter consistently decreased with increasing Cd concentration (Hakla et al., 2021).

Lv et al. showed that Cd stress reduced the biomass of above-ground and root tissue of rice, the stem fresh weight of rice cultivar NJ6 in the 10 μM Cd and 50 μM Cd groups decreasing by 59,6% and 42,3%, respectively, and that of ‘Y32’ decreasing by 39,5% and 36,8%, respectively, relative to no-Cd control plants. (Lv et al., 2019a)

Cd treatments reduced the growth attributes of 20 days old seedlings of two sunflower varieties. The high concentration of 150- and 200-mM Cd and Pb drastically reduced Mn and K contents, vigor, length, and biomass. Thus, Cd was found more toxic than Pb and Ni (Sadiq et al., 2019).

Regulation of crop photosynthesis and respiration under Cd stress. Khanboluki et al. found that Cd concentration increasing in soil not only decreased the dry weight of root and shoot of wheat and sorghum, but also decreased many other plant growth indicators, such as leaf area, chlorophyll a, b and carotenoid concentrations. (Khanboluki et al., 2018)

Kanu et al. also pointed out that Cd toxicity in rice led to a substantial reduction in chlorophyll (Chl) a, Chl b and carotenoid concentrations. Under 100 mg/kg $CdCl_2 \cdot 2,5\ H_2O$ concentrations, the maximum reductions in chlorophyll a, b and carotenoid concentrations, relative to the control, were 12,21%, 56,17% and 27,32%, respectively, for rice ‘Guixiangzhan’ and 31,43%, 32,74% and 68,33%, respectively, for ‘Meixiangzhan 2’. (Kanu et al., 2019a)

Zhou et al. studied two wheat varieties differed in grain Cd accumulation ability under 10 μM Cd stress and found that Cd stress significantly reduced net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and particularly transpiration rate (Tr), as well as significantly inhibiting root-growth, from 12,9% to 33,7%. (Zhou et al., 2019b)

Increasing Cd concentration in the soil reduced growth parameters, Chl a, and Chl b contents, as well as Fv/Fm and ETR (electron transportation rate) values but increased root, stem, and leaf Cd accumulation and the proline content in sunflower (Shahabivand et al., 2017).

Regulation of crop protein and hormone under Cd stress. Plants cope with the adverse effects of stress by adjusting their protein metabolism and plant hormone levels and balance. Total protein and flavonoid contents of plants grown in a Cd-contaminated environment were significantly lower in Cd-stressed in maize plants than in the control plants (Abbas et al., 2020). Total soluble sugar concentration also decreasing in response to Cd stress (Hakla et al., 2021).

Phytohormones play an important role in the control of induced defences against Cd stress via the regulation of levels of gibberellins (GA), salicylic acid (SA), jasmonates (JA), abscisic acid (ABA), indoleacetic acid (IAA) and ethylene (ET) (Kim et al., 2016).

Lin et al. studied plant hormone changes in response to Cd uptake in rice and found that all rice accessions tested showed significant increases in SA and ABA in response to Cd stress (50µM Cd), while JA and IAA levels decreased, compared with the no-Cd control plants (Lin et al., 2018).

Cd accumulation and antioxidant reaction. Plants respond to Cd stress though adjusting their own physiological and biochemical processes, of which the accumulation and subsequent detoxification of reactive oxygen species (ROS) caused by heavy metals is one of the important aspects (Abbas et al., 2020; Chen et al., 2010a; Jan et al., 2021; Saidi et al., 2021; Wu et al., 2015b; Zhang et al., 2020b). Cd stress disrupts the dynamic balance between production and quenching of ROS in plants (Lv et al., 2017). Excessive ROS accumulation changes enzyme activity disrupts the metabolism of proteins, lipids and nucleic acids results in damage to membrane lipids by peroxidation and inhibits plant growth and development (Christophe et al., 2017; Dixit et al., 2001; Hu et al., 2019a).

To reduce the oxidative damage caused by the excessive ROS induced by Cd stress, plants have evolved antioxidant enzyme and non-enzyme systems during the long-term phylogenetic process (Liu et al., 2022). Antioxidant responses to Cd stress have been studied in many plant species, such as sunflower (Gopal & Nautiyal, 2011; Saidi et al., 2021), soybean (Li et al., 2012b), wheat (Chen et al., 2017a), barley (Chen et al., 2010b), rice (Singh et al., 2020), maize (Rehab & Ibrahim, 2020), rapeseed (Wu et al., 2015c) and millet (Han et al., 2018c).

However, there have been few studies on antioxidant responses directly related to Cd accumulation in cereal grains. Chiao et al. reported that genotypic differences in

Cd distribution in rice plants and subsequent Cd accumulation in grains may be due to physiological regulation of Cd toxicity tolerance (Chiao et al., 2019b).

Hassan et al. compared the antioxidant enzyme activities of two rice varieties exhibiting large differences in grain Cd accumulation (Hassan et al., 2005). Generally, the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) decreased with increasing Cd concentration, whereas the concentration of malondialdehyde (MDA), a marker of membrane lipid peroxidation, increased with increasing Cd concentration. In response to increasing Cd concentration in the nutrient solution, MDA concentration in shoots and roots of the high-Cd cultivar increased at a much higher rate than did that of the low-Cd cultivar. So the high-Cd cultivar was more sensitive to Cd than was the low-Cd cultivar.

Shi et al. studied two other rice cultivars with different grain Cd concentrations (Shi et al., 2013). The results showed that the high-Cd cultivar had greater Cd tolerance, whereas the synergistic effects of the antioxidant enzyme systems (SOD, CAT and POD) in the plant tissues were higher in the high-Cd cultivar than those in the low-Cd cultivar. Furthermore, the MDA concentration in leaves of the high-Cd cultivar was significantly lower than that of the low-Cd cultivar, and the degree of both cell membrane peroxidation and subsequent damage were also lower.

Another study showed that Cd stress reduced antioxidant enzyme activities of SOD, POD and CAT, increased the concentrations of H_2O_2 and MDA, and increased oxidative damage, whereas calcium treatment could alleviate the degree of oxidative damage and significantly reduced the concentration of Cd in grains (Kanu et al., 2019b).

Khan et al. studied the effects of Cd-contaminated soil on wheat physiology, and showed that Cd concentration in the grains was positively correlated with the H_2O_2 and MDA concentrations in wheat, and negatively correlated with plant biomass, chlorophyll concentration, and the activities of SOD and POD (Khan et al., 2020a).

1.4 Molecular mechanism of Cd accumulation in grains

Over the past 40 years, the rapid development of molecular marker technology and high-throughput sequencing platforms has greatly promoted the study of quantitative trait loci (QTLs). With the continuing progress with respect to science and technology, there have been many reports of QTLs related to Cd accumulation in plants (Glenda et al., 2010; Kouichi et al., 2010; Xu et al., 2012; Zvonimir et al., 2014).

The identification of QTLs related to Cd accumulation in grains has attracted increasing attention, until it is now one of the most important research fields of molecular breeding, with the aim of selecting for cultivars which accumulate low levels of grain Cd.

Identification of QTLs for regulating Cd concentration in grains by linkage mapping. At present, there are few reports on the research and application of linkage mapping on Cd accumulation in cereal grains, but some achievements have been made. Using 39 chromosome fragment substitution rice lines from the *O. indica* landrace Kasalath and the Japanese premium *O. japonica* cultivar, Koshihikari. Ishikawa et al. analyzed QTLs for Cd accumulation in rice for the first time, and identified QTLs for controlling Cd concentration on chromosomes 3,6 and 8 in brown rice. The Cd uptake and translocation from root to the shoots are important factors affecting Cd accumulation in grains (Ishikawa et al., 2005).

Using 146 F_2 generation populations derived from a cross between high- and low-Cd rice parents with a 13-fold difference in Cd concentration in the stem, Ueno et al. detected a major QTL on chromosome 11 for translocating Cd from the root to above-ground tissue, with the corresponding marker RM6623 explaining 16,1% of the phenotypic variation in Cd accumulation (Ueno et al., 2009).

Ishikawa et al. established 85 backcrossed recombinant inbred lines (RIL) from a cross between two parents, namely a low Cd- accumulating *O. japonica* variety and a high Cd-accumulating *O. indica* variety, one QTL for Cd accumulation in grains, *qGCD7*, was detected on the short arm of chromosome 7, which explained 35,5% of the phenotypic variation in Cd concentration and played an important role in controlling Cd concentration in grains (Ishikawa et al., 2010).

Using the RIL $F_{6:8}$ population from high- and low-Cd soybean parents, Jegadeesan et al. performed QTL analysis, using a linkage map established by 161 simple sequence repeat (SSR) markers; a major QTL for low Cd accumulation in grains was located by the flanking markers SatK 113 and SatK 58 on the linkage group LG-K, the same position as *Cda1,* which explained 57,3% of the phenotypic variation (Jegadeesan et al., 2010).

Eduardo et al. established a population produced from crossing two soybean parents with different Cd concentrations, and detected a major QTL for Cd accumulation in the grains (SSR markers *GM09:4770663* and *GM09:4790483*), located on chromosome 9, which was stably inherited over several generations, explaining phenotypic variation in the three generations of 82, 57 and 75%, respectively (Eduardo et al., 2010). Wiebe et al. carried out expressed sequence tag markers (ESEs) and sequence tagged site (STS) analysis in 155 doubled haploid (DH) populations from the cross W9262-260D3 (low Cd) by Kofa (high Cd), and revealed two ESMs and one STS, and the data explained >80% of the phenotypic variation for grain Cd concentration (Wiebe et al., 2010).

Hideki et al. using 126 RIL populations raised from a cross between high yielding Japanese rice and African low-Cd upland rice, detected a QTL (*qLCdG11*) for low Cd accumulation located on chromosome 11 in 2008 and 2009, which explained 9,4% and 12,9% of the phenotypic variation, respectively, in the two study years (Hideki et al., 2011).

Oladzad-Abbasabadi et al. identified single nucleotide polymorphism (SNP) markers *IWB55063* and *IWB47298* associated with a novel low Cd uptake QTL site in durum wheat strain D041735, located on the long arm of wheat chromosome 5B, the markers being effective for the identification of high- and low-Cd materials (Oladzad-Abbasabadi et al., 2018). Guo et al. identified, from a F_2 population, two new markers, *qBRCdC-9* and *qBRCdC-12*, alleles of YaHui2816, for a Cd accumulation QTL in brown rice, which could effectively reduce the Cd concentration in brown rice in different genetic backgrounds (Guo et al., 2019).

Wang et al. investigated a number of QTLs controlling element concentration in grains derived from a rice RIL population over a three-year study period conducted at three different locations, and located five QTLs for Cd grain accumulation, which explained 5,09-10,53% of phenotypic variation in Cd concentration (Wang et al., 2020b).

Identification of QTLs regulating Cd concentration in grains by correlation mapping. Wu et al. performed association analysis on Cd accumulation in different organs from plants of 100 core barley accessions, and found marked genotype differences in Cd concentrations in all organs, with Cd concentrations in grains being associated with those in stems, but not with those in roots; 15 QTLs for Cd accumulation in grains were detected, with the two main QTLs being located on chromosomes 2H and 5H, respectively (Wu et al., 2015a).

Zhang et al. carried out a genome-wide association study (GWAS) on 698 germplasm accessions from *indica* and *japonica* subpopulations. Sixty-two loci related to grain Cd concentration were located in 29 QTL clusters, according to their physical locations, among which the markers *qCd4-7, qCd6-2 and qCd8-1* had the characteristics of stable expression and/or expression independent of genetic background and had high application value in breeding (Zhang et al., 2018a).

Zhao et al. identified 312 different populations screened for Cd accumulation from 1568 rice accessions, and identified seven QTLs for low Cd accumulation in *indica* and *japonica* subpopulations, respectively, using the GWAS technique (Zhao et al., 2018). Liu et al. conducted GWAS analysis of heavy metal concentrations in grains based on 276 rice accessions, using 416K SNPs, and detected 17 QTLs for altered Cd accumulation in grains (Liu et al., 2019c).

Pan et al. identified 35 QTLs significantly associated with Cd accumulation over two years through SNP sequences and GWAS, based on 338 rice varieties; the marker *qCd8-1* was detected in both years of the study, and nine QTLs were the same as the identified genes or QTLs (Pan et al., 2020b).

Ban et al. conducted association analysis for Cd concentration in grains of 43 populations derived from doubled haploids from the F_1 generation of a cross between a high- and a low-Cd common wheat. Two new locus, controlling low Cd concentration in grains, was found on the short arm of chromosome 4B and the long arm of chromosome 6B, explaining 9,4%–25,4% (4B) and 9,0%–17,8% (6B) of the phenotypic variation in grain Cd concentration, respectively (Ban et al., 2020).

The results of QTL identification associated with altered Cd accumulation in grains provides the basis for further isolation of new Cd accumulation-related functional genes and the subsequent development and cultivation of low Cd-accumulation-related varieties through marker-assisted breeding.

Cloning and functional analysis of genes related to Cd accumulation in grains. Identifying genes associated with low Cd accumulation and studying the mechanisms of crop uptake and transport to achieve low Cd accumulation in the grains are steps of great significance in the development and cultivation of low-Cd crop varieties (Chen & Wu, 2020).

Cd transporter proteins have been identified in rice and other cereal crops by studying genotypes, including mutants, with different levels of Cd accumulation. Related genes involved in the regulation of Cd accumulation have been found, such as the natural resistance-associated macrophage protein gene (*NRAMP*) (Takahashi et al., 2011), heavy metal ATPases gene (*HMA*) (Takahashi et al., 2012a)a, Zn/Fe-regulated transporter-like gene (*ZIP*) (Guerinot, 2000b; Zheng et al., 2018b) and low affinity cation transporter gene (*LCT*) (Shimpei et al., 2011).

Although significant progress has been made in the identification of QTLs/genes related to Cd accumulation in rice, our understanding is still very limited (Zhao et al., 2018).

OsHMA3, expressed in root cell vacuoles, is the first gene responsible for Cd accumulation in rice grains to be cloned; it restricts Cd translocation from roots to above-ground tissues by selectively isolating Cd into root vacuoles (Ueno et al., 2010). The Cd accumulation trait in durum wheat is controlled by the major locus *Cdu1* (Clarke et al., 1997), which is perfectly matched with the *HMA3-B1* gene located on chromosome arm 5BL (Knox et al., 2009). *OsHMA2*, a homologous gene of *OsHMA3*, is localized in the pericycle of roots and the phloem of diffuse vascular bundles of nodules (Yamaji et al., 2013), is the main transporter of Zn and Cd from the root to the shoot. The studies have shown that mutation induction or the regulation of expression of *OsHMA2* can reduce Cd concentration in grains (Satoh-Nagasawa et al., 2012; Takahashi et al., 2012c).

Liu et al. found that sequence variation in the *OsHMA3* promoter GCC7 was an important factor in controlling differences in Cd accumulation between *indica* and *japonica* accessions (Liu et al., 2020c).

High and low Cd accumulation alleles, *GCC793-11* and *GCC7PA64s*, are preferentially distributed in *indica* and *japonica*, respectively, and the two *GCC7* alleles have different *OsHMA3* initiation activities, resulting in different Cd concentrations in grains of the two subspecies. Expression of *OsHMA3* under the control of the *OsHMA2* promoter can store more Cd in vacuoles of different tissues, effectively reducing Cd accumulation in rice (Shao et al., 2018).

Mutations of the metal transporter gene *OsNramp5* can significantly reduce Cd uptake by roots, leading to a decrease in Cd concentration in rice grains (Satoru et al., 2012). A new *indica* cultivar with low Cd accumulation was obtained through *OsNramp5* knockout by the CRISPR/Cas9 gene editing system, with Cd concentration in the grains of less than 0,05 mg/kgbeing stable, and the yield not being significantly affected when grown in a Cd-contaminated paddy field (Tang et al., 2017).

Another gene in this family, *OsNRAMP2*, may encode a functional Cd transporter, with subcellular localization analysis showing that *OsNRAMP2* was expressed in vacuoles and was considered to be a new functional candidate gene associated with Cd accumulation in rice (Zhao et al., 2018).

The low cadmium (LCD) protein mainly distributed in the cytoplasm and nucleus, and the gene is expressed in vascular tissues of roots and in phloem-associated cells of leaves; The Cd concentration in rice grains of the Cd-tolerant *lcd* knockout mutant decreased by about half compared with the wild type, but there was no significant difference in plant biomass or yield from the values of the wild type when both genotypes were grown under low Cd soil conditions (Hugo et al., 2011).

The Ca^{2+} cation exchanger gene *OsCCX2* is another newly discovered rice gene that can mediate the direct transport of root-derived Cd to grains, and the knockout mutant of the *OsCCX2* gene significantly reduced the grain Cd concentration in rice (Hao et al., 2018).

Guo et al. studied the Cd distribution in YaHui2816, a cultivar with low Cd in the grain and high Cd in the stem, the results showing that *OsHMA2*, *OsCCX2*, *OsZIP7* participated in the process of Cd retention in node II, reducing the Cd transport to grains, opening up the possibility of YaHui2816 having potential for both safe food production and Cd soil phytoremediation (Guo et al., 2020).

1.5 Effects of exogenous factors on Cd accumulation

Cd in plants is partially accumulated in the edible parts of plants or grains by deposition from the atmosphere (onto leaves) or in the soil (through the roots). It then enters the human body through the food chain (Feng et al., 2011). Studies was carried out in an industrial park of Hunan Province (China) and it was shown that the average contribution rates of three possible pollution sources, namely industrial dust, agricultural fertilizer and automobile exhaust fumes, to Cd content in rice grain were 87%, 9% and 4%, respectively (Yan et al., 2021b). Therefore, in grain-producing areas, we should not only pay attention to soil remediation, but also strengthen the supervision of industrial waste discharge from nearby factories and mines, strengthen the comprehensive control and prevention of air pollution, and improve the capacity of urban sewage collection and industrial wastewater disposal from industrial parks to strictly control and eventually eliminate the sources of soil and air pollution.

Appropriate use of traditional agricultural planting methods in Cd-contaminated farmland can not only make use of Cd-overaccumulated plants to remediate the soil, but also produce crops, which meet the national Cd limit standard (El-Hassanin et al., 2020; Kang et al., 2020; Li et al., 2017; Liu et al., 2016; Song et al., 2013; Yan et al., 2021a).

By properly managing soil moisture and nutrients, and by controlling soil pH and redox potential, farmers can reduce Cd migration from soil to root, helping to decrease Cd accumulation in grains (Hussain et al., 2021; Yuan et al., 2020).

Water and fertilizer management has shown some positive effects in reducing the availability of heavy metals in paddy soils (Belhaj et al., 2016; Grant et al., 2013; Murtaza et al., 2015; Zhu et al., 2020); flooding can reduce Cd accumulation in rice, while adding lime can have a similar effect (Han et al., 2018a). Although it has been reported that flooding may increase the accumulation of As in crops (Hu et al., 2013).

Wang et al. showed that film mulch technology could reduce Cd content in rice by 50% compared with the control; when combined with other measures (biochar + silica foliar fertilizer), Cd content in grains could be reduced even further (Wang et al., 2015). At present, the effects of factors, applied singly or in combination, on the control of Cd pollution are being studied (Ashrafi et al., 2015; Monu et al., 2008; Tang et al., 2020; Zhou et al., 2020b), but such studies have not been sufficiently systematic. Due to the complexity and diversity of crop Cd pollution sources, further research still needs to be carried out.

The rational utilization of fertilizers and conditioners can improve the physical and chemical properties of soil, reduce plant Cd uptake from the soil and promote plant growth (Chen et al., 2021b; Jan et al., 2021).

Grain Cd accumulation is closely related to the bioavailability of Cd in the soil; over a certain range of soil pH, soil acidification increases the content of available Cd in soil, leading to increased Cd accumulation in grains (Chen et al., 2021a; Zhang et al., 2021c).

Alkaline soil amendments, such as lime, zeolite or sepiolite, can achieve soil Cd complexes, chelates, and precipitates, thus, reducing the bioavailability of Cd in soil (Bashir et al., 2021; Hamid et al., 2019; Jin et al., 2020). Huang et al. used quicklime for four consecutive years to remediate acid farmland polluted by Cd. The results showed that the average soil pH increased by 0,57, exchangeable or water-soluble Cd components in the soil decreased by 17%, organic binding of Cd components increased by 10%, and the grain Cd concentration of crops dropped below the threshold value of 0,2 mg/kg (Huang et al., 2020).

The concentration of Cd in the leaves and receptacles of sunflower plants increased with biochar application but their concentration in the roots, stems and seeds significantly decreased compared with the control. The total amount of accumulated Cd in sunflower plants increased by 15,8-42,3% compared with that in the control (Jun et al., 2020). A range of chemical regulators have been used to reduce plant Cd uptake, such as engineered nanoparticles (Fox et al., 2020), salicylic acid ((Snehalata et al., 2020; Wang et al., 2021a) and melatonin (Lv et al., 2019b).

Chelators EDDS/CA (trisodium (S,S)-ethylenediamine-N,N'-disuccinic acid/citric acid) significantly increased Cd accumulation but inhibited the plant growth of sunflower and, in contrast, ALA (5-aminolevolinic acid) promoted both Cd absorption and biomass accumulation especially when applied in combination with EDDS (Xu et al., 2021).

Sarwar et al. pointed out that spraying $ZnSO_4$ solution at an appropriate concentration onto the wheat leaf surface at the booting stage could effectively improve the adverse effects of Cd on wheat grains in crops grown in Cd-contaminated soil and reduce the Cd concentration in wheat grains (Sarwar et al., 2015).

Membrane integrity due to lipids proved that SA could be used as a potential growth regulator and a stabilizer of protection of Cd-induced oxidative stress to improve sunflower resistance to Cd stress (Sakineh et al., 2012).

Evidence has shown that low molecular weight organic acids *(LMWOA*), which are involved in heavy metal resistance mechanisms in plants with the addition of malic (*MA*) or acetic (*AA*) acids increased endogenous accumulation of *LMWOA*, especially in the roots, which could be beneficial for sunflower metabolism (Hawrylak-Nowak et al., 2015). *LMWOA* can increase Cd accumulation in plants. Thus, a lower application rate (2mmol kg^{-1}) and an earlier application date (20 days after seedling emergence) are beneficial for improving the phytoremediation efficiency of sunflowers. The increase in root biomass expanded the contact area between plants and soil, which was one of the reasons for the increase in Cd accumulation (Lu et al., 2021a; Lu et al., 2021b).

The isolation and identification of Cd-tolerant microorganisms from Cd-contaminated soil, and evaluation of the potential of plant–microbial symbiosis to achieve Cd remediation may provide effective ways of achieving soil bioremediation and reducing Cd accumulation in grains (Adewole et al., 2010; Pathom-Aree et al., 2021; Saghir et al., 2020).

Wang et al. found that treatment of Cd-contaminated soil with *Bacillus cereus* strain M4 fermentation broth could promote the growth of potted rice seedlings growing in Cd-contaminated soil, while the Cd concentration in rice grains decreased from 0,309 to 0,186 mg/kg (Wang et al., 2019a).

Arbuscular mycorrhizal fungi grow naturally in plant roots, promoting plant growth, and play an important role in conferring heavy metal tolerance (Garg & Bhandari, 2014).

Li et al. found that arbuscular mycorrhizal fungi could reduce Cd accumulation in the maize plant, but could be harmful to maize seedlings, so that the selection and utilization of microorganism species and strains for soil remediation need further exploration (Li et al., 2020).

1.6 Breeding of low Cd varieties

Cd accumulation in cereal grains is a serious threat to food safety and human health. Cultivating low-Cd crop varieties is one of the most effective ways to reduce Cd toxicity (Grant et al., 2008; Ishikawa, 2020; Liu et al., 2020b; Sun et al., 2015; ZaidImdad et al., 2018). Low-Cd crop breeding technology presents a developmental trend from conventional breeding to the combination of conventional and molecular breeding and from empirical breeding to precision or design breeding.

Attempts have been made to reduce Cd accumulation in grains by manipulating the Cd transporters through overexpression or knockout of the transporter genes, as well as through marker-assisted selection breeding, based on genotypic differences in Cd accumulation in grains (Ma et al., 2021).

Cultivation of various low Cd crops. At present, various crops such as rice (Huang et al., 2021; Pan et al., 2020a; Yan et al., 2021a), maize (Dakak & Hassan, 2020; El-Hassanin et al., 2020; He et al., 2017), wheat (Ali et al., 2021; Khanboluki et al., 2018; Wang et al., 2020a), and cotton (Zhu et al., 2020) have been studied for their Cd tolerance mechanism and low Cd material screening.

Different crops or varieties have different Cd grain accumulation abilities. A study showed that *indica* was easier to accumulate Cd than *japonica*, and the Cd content in grains of *indica* rice was 1,84-4,14 times higher than *japonica* (Kun et al., 2019).

The Cd content of *indica* varieties may be higher than the national Cd limit under medium and low Cd-contaminated soil conditions (Chen et al., 2019).

Chen et al. separately introgressed one gene, *OsHMA3*, and one QTL, *qlGCd3*, related to low Cd accumulation, into the recipient parent C5S by molecular marker-assisted breeding and obtained improved material with the low-Cd trait expressed consistently, with the average Cd concentration of the improved material carrying *OsHMA3* or *qlGCd3* reduced by 52,8% and 50,8%, respectively, compared with that of wild-type ‘C5S’ (Chen et al., 2020).

OsNRAMP5 is the main transporter of Cd and the essential element manganese (Mn) in rice plants. Yang et al. knocked out the function of *OsNRAMP5* using the CRISPR/Cas9 gene editing technique in two *japonica* rice cultivars. In paddy field experiments, these loss-of-function *OsNRAMP5* mutants significantly reduced Cd concentration in grains, but Mn accumulation was also significantly decreased, which affected plant height, seed setting rate, grain number per panicle and other agronomic traits, leading to a slight decrease in crop yield. In addition, it has been reported that Cd concentration in plants is positively correlated with yield, which brings difficulties and challenges to breeding research (Yang et al., 2019). It is believed that the following problems still remain in breeding for low Cd varieties.

Firstly, current basic research is out of line with breeding applications, and many newly discovered markers and genes related to Cd accumulation have not been used in germplasm innovation through transgene transformation or gene editing.

Secondly, there are few molecular markers or Cd regulatory genes with significant practical value, so it is difficult to meet the food safety needs by biotechnological breeding approaches, such as molecular design.

Gene manipulation techniques and their application to new cultivar development should be treated with caution and evaluated comprehensively. Many studies have shown that there are antagonistic effects on uptake between Cd and mineral elements during plant growth and development (Hou et al., 2021; Jia et al., 2016).

Cultivation of low Cd-accumulation sunflower varieties. Sunflower （*Helianthus annuus* L.） belongs to the family of Asteraceae (Frey et al., 2020; Zhang & Elomaa, 2021). The *Helianthus* genus contains 65 different varieties, 14 of which are annual plants. The sunflower that most people refer to is *H.annuus*, an annual sunflower. The plant has a rough, hairy stem, broad, coarsely toothed, rough leaves, and circular heads of flowers. The heads consist of many individual flowers which mature into seeds on a receptacle base. Sunflower is the world's fourth largest oil-seed crop, and its seeds are used as food and its dried stalk as fuel. It is also used as an ornamental plant, too (Benavides et al., 2021; Zhao et al., 2011). Sunflower has a large biomass and shows high tolerance to heavy metals and therefore, are used in phytoremediation studies (Bayat et al., 2021a; Benavides et al., 2021; Chae et al., 2014; De Andrade et al., 2018; Tang et al., 2003; Watai et al., 2004).

Cultivating low Cd-accumulation sunflower varieties is the fundamental method to solve the problem of low Cd intake. The cultivating process is long and complicated.

The cultivating process probably includes:

1) Finding materials with Low Cd genes;

2) Finding materials with high and stable yield, resistance to disease and insect pests, wide adaptability, and other high-quality materials except for low Cd characteristics. These materials should be the main varieties, considering other excellent characteristics such as herbicide tolerance, drought and flood resistance, maturity period, and so on;

3) To understand the genetic characteristics of the Cd gene, and to formulate the low Cd hybridization cultivation strategy assisted by modern biotechnology;

4) Evaluate and select hybrid offspring materials and select new varieties (lines)

with low Cd, high quality, and good comprehensive characteristics;

5) Experimental adaptive planting of new varieties (lines), observation, and evaluation of traits stability.

As the main grain crops, such as rice, wheat, corn, and soybean, there are many studies on the cultivation of varieties with low Cd. But there are few reports on the cultivation of varieties with low Cd in sunflowers. Li et al. screened 200 germplasm resources and selected two varieties with low Cd accumulation – Primrose and HA290, and two maintain lines – HA323 and RHA324 with medium content of Cd (Li et al., 1995).

Two new low Cd varieties of HA448 and HA449 were selected by screening the later generation of HA323/HA290. RHA324/Primrose was selected as the RHA450 recovery line. The average Cd content of HA448/RHA450 and HA449/RHA450 hybrids was reduced by more than 50% in the three-year experiment from 2000 to 2002 (Miller et al., 2005). Then, he investigated the variability of grain Cd levels of sunflower by field experiment and sought an efficient screening method for future cultivation, The result showed large variations in leaf Cd concentration among 200 sunflower lines. The positive correlation between R5 leaf Cd and kernel Cd level was obtained from a non-oil-seed hybrid. It indicates that an efficient and low-cost screening method can be developed for genotype selection, but plants must be grown to the R5 stage.

Are there any excellent characteristics of the new breeding materials, such as low grain Cd concentration, stability, and acceptability in the move from the laboratory to the test plot and then to the field? Is there an optimal planting region for the low Cd varieties identified by trialing throughout the growing regions? Consequently, gene manipulation techniques (e.g., transformation or gene editing) and their application to new variety development should be treated with caution and evaluated comprehensively and systematically. Whether such new low Cd varieties can be successfully developed by plant selection breeders and would have the characteristics allowing them to be widely adopted by farmers, has yet to be confirmed.

Conclusions to Chapter 1

Therefore, it is necessary to strengthen basic research into the regulation of Cd accumulation by systematically analyzing the molecular basis of agronomic traits associated with Cd accumulation, exploring the available excellent gene resources, innovating, and integrating conventional breeding, molecular design breeding, and crop breeding information technology, and establishing an efficient and rapid selection system.

CHAPTER 2

CONDITION, MATERIAL AND METHODS OF RESERCH

2.1 Materials of the research

The study of the sunflower collection was carried out in 2 stages. The first – in terms of the vegetation experiment –104 samples （Table 2.1) and the second – in terms of the field experiment – 95 samples.

Table 2.1

Sunflower test material

N0.	Variety	No.	Variety	No	Variety	No.	Variety
1	Chas	27	58\2	53	S2K670	79	UE0100243
2	Esman	28	63\1	54	ДН-47-9	80	UE0100274
3	Oniks_2007	29	57\2	55	Rf 1407/1054	81	UE0100711
4	Syaivo_1	30	59\1	56	M1049	82	UE0100712
5	Syaivo	31	56\3	57	МВГ2	83	UE0100713
6	Neon_2011	32	62\3	58	J01	84	UE0100714
7	Teo	33	62\3	59	APS lider	85	UE0100715
8	Oniks	34	63\3	60	КГ49	86	UE0100938
9	Sumchanyn	35	Huslyar	61	UE0100018	87	UE0100939
10	66\1	36	Amis CL	62	UE0100026	88	UE0100956
11	69\1	37	Polaris CL	63	UE0100035	89	UE0100964
12	71\1	38	Rezon	64	UE0100039	90	UE0100968
13	Jan-67	39	Serpanok	65	UE0100041	91	UE0100977
14	54\1	40	P64LE99	66	UE0100043	92	UE0100981
15	55\2	41	PR63LL01	67	UE0100044	93	UE0101021
16	52\2	42	APS49	68	UE0100045	94	UE0101082
17	64\3	43	Snk103	69	UE0100047	95	UE0101177
18	65\2	44	424924p7-4	70	UE0100051	96	UE0101323

19	59\2	45	Z1064	71	UE0100052	97	P64LC108
20	59\3	46	Snk630	72	UE0100056	98	Tutti
21	68\1	47	J071/1	73	UE0100060	99	Sumico
22	64\2	48	DN47-2	74	UE0100077	100	STH-17112
23	57\1	49	МВГ3	75	UE0100084	101	STH-12003
24	61\1	50	M1043	76	UE0100114	102	STH-12005
25	66\1	51	JB231AC	77	UE0100118	103	P64HE118
26	65\1	52	JG3	78	UE0100238	104	STH-16004

Selection samples for the research were provided by educational and scientific institutions of Ukraine according to the agreements with determined regulations of use. The seeds of commercial sunflower hybrids and varieties were purchased from specialized seed stores.

2.2 Vegetation, field, and laboratory experiments

2.2.1 Vegetation experiment on testing sunflower samples

The substrate for growing the plants was prepared from a peat and sand mixture in a volume ratio of 4:1. Cadmium was added to the substrate during its preparation in the form of an aqueous solution of cadmium monosulfate. The source of cadmium is $CdSO_4$. Cadmium concentration in the substrate at the time of seed sowing was 1,0 ± 0,03 mg/kg in the background.

Vegetation of plants took place in 1,2-liter plastic pots. Three plants were growing in one pot. The temperature was maintained at the following level: daytime –18-22 °C; night – 16-18 °C. Lighting was natural + artificial with a total duration of 12 hours. The vegetation period was 54–75 (before the R5 phase). After the R5 phase started, the aerial part of the plants was cut and dried to an air-dry state. The weight of one plant was determined. The sample for cadmium determination was prepared from 3 plants.

2.2.2 Field experiment on the collection study

Collection testing of the selection samples and inter-varietal hybrids was carried out in terms of the field experiment. The plots were in 2 rows with a row spacing of 0,7 m and a length of 9,0 m. The distance between plants in a row was 0,23 m. The estimated density was 60,000 plants/ha. The plot area was 12,6 m^2. The repetition is 3 times. The placement of plots in repetitions was randomized. Vegetation: Stairs – R10. The natural background of cadmium in the soil was 0,21± 0,01 mg/kg.

Sowing in the plots was carried out with manual planters in the last decade of April. Crop care included the removal of double sprouts and treatment of areas with anti-cereal herbicides. Collection and threshing of inflorescences were carried out manually.

The outermost plants in the row were not threshed. Inflorescences under insulators were collected and threshed individually after the additional drying. The determination of the metric and weight parameters of plants was carried out in the flowering phase. Under laboratory conditions, the following parameters were evaluated: weight of 1000 seeds, huskiness of the seeds, oil content in the seeds, and cadmium content in the seeds (Volkodav et al., 2000).

The crossing was carried out using insulators made of agricultural fiber (spunbond, 42 g/m^2). Isolation of the inflorescence was carried out on the first and second days of flowering with the removal of rows of reed flowers and opened tubular flowers. Pollination was carried out twice on the 3rd and 5th day after the isolation.

After the end of the growing season, the inflorescences were cut and dried (in insulators) until technological maturity . Threshing of heads and seeds sorting was done manually. Samples with a low level of seed formation (less than 10 pcs/inflorescence) were . discarded.

Crossing schemes are presented in Tables 2.2 and 2.3.

Table 2.2

Cross combinations between low cadmium varieties

Varieties		**17**	**31**	**37**	**38**	**46**	**51**	**53**	**61**	**86**	**99**
17	64\3		X	X	X	X	X	X	X	X	X
31	56\3	X		X	X	X	X	X	X	X	X
37	Polaris CL	X	X		X	X	X	X	X	X	X
38	Rezon	X	X	X		X	X	X	X	X	X
46	Snk630	X	X	X	X		X	X	X	X	X
51	JB231AC	X	X	X	X	X		X	X	X	X
53	S2K670	X	X	X	X	X	X		X	X	X
61	UE0100018	X	X	X	X	X	X	X		X	X
86	UE0100938	X	X	X	X	X	X	X	X		X
99	Sumico	X	X	X	X	X	X	X	X	X	

Table 2.3

Cross combinations between high cadmium varieties

Varieties		**33**	**41**	**52**	**71**	**72**	**73**	**82**	**85**	**90**	**104**
33	62\3		X	X	X	X	X	X	X	X	X
41	PR63LL01	X		X	X	X	X	X	X	X	X
52	JG3	X	X		X	X	X	X	X	X	X
71	UE0100052	X	X	X		X	X	X	X	X	X
72	UE0100056	X	X	X	X		X	X	X	X	X
73	UE0100060	X	X	X	X	X		X	X	X	X
82	UE0100712	X	X	X	X	X	X		X	X	X
85	UE0100715	X	X	X	X	X	X	X		X	X
90	UE0100977	X	X	X	X	X	X	X	X		X
104	STH-16004	X	X	X	X	X	X	X	X	X	

Inheritance coefficients and indicators of the degree of phenotypic dominance were determined by the method of B. Griffing (Griffing. 1950). The assessment of the range of dominance was carried out according to the scale of G. M. Beil, R. E. Atkins (Beil & Atkins. 1967) where the numerical values corresponded to the scheme: The analysis of the degree of phenotypic dominance in the selections of growing components was carried out to quantify the manifestation of traits for the rapid assessment of hybrid offspring and the improvement of traits.

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

In field experiments on evaluating the values of intervarietal hybrids, the result was compared to a conditional standard. Conditional standard - average values of indicators of hybrids: Rezon, Polaris CL, Sumico.

2.2.3 Laboratory studies

Determination of cadmium content. Vegetative organs and plant seeds were dried to an air-dry state. The samples were ground on a cadmium-free mill followed by acid mineralization. For autoclave acid mineralization, a weight of a suitably prepared crushed plant sample weighing 0,100±0,005g was taken, transferred to an autoclave, and moistened with 0,5 ml of bidistilled water. Then, 1,5 ml of nitric acid was added, and decomposition was carried out for two hours at a temperature of 200 ^{o}C. After cooling to room temperature, the contents were transferred to a measuring tube and made up to 10 ml with bidistilled water.

To prepare the calibration solution, standard samples of aqueous solutions of metal salts were used from DSZ RM-28 022.127-00 (Ukraine). All solutions were prepared in bidistilled water with an electrical conductivity of no more than 1 μS.

Statistical processing of experimental data for the generalization and definition reliability of the obtained results of studying the variability of productivity morphological and physiological parameters was carried out by using variation, dispersion, and correlation analysis according to standard methods using MS Excel 2010 software and Statistica (Carenko et al., 2000).

Screening conditions of two sunflower varieties with high/low Cd accumulation. Accessions of two sunflower genotypes' of JB231AC and 62\3, which were determined with different Cd accumulation capabilities in our previous study (data unpublished) were carried out in this study. The two varieties were obtained from the National Agrarian University in Sumy, Ukraine where they were reproduced in May 2019.

In 2020, the healthy seeds of each accession were sterilized with 15% H_2O_2 for half an hour and rinsed with distilled water at least three times, soaked in deionized water at room temperature for four hours, then sown in the germination box (32cm×25,5cm×11cm) containing vermiculite, and moistened with the deionized water. Seeds were incubated in the culture room with 16h light (28 °C, 5000Lux) and 8h dark (25 °C) photoperiods for 6 days. Following the germination, seedlings were transferred to plastic pots (19cm×13cm×12cm) filled with 10L of 1/4 strength modified Hoagland nutrient solutions for 7 days, which then was increased to 1/2 strength for 7 days. After 20 days of Cd-free growth, seedlings with uniform sizes were randomly assigned to four different Cd treatments: 0, 25, 50, and 100µM $CdCl_2 \cdot 2.5H_2O$ for 7 days. The 1/2 strength nutrient solution contained 2,5mmol/L $Ca(NO_3)_2$, 1mmol/L $MgSO_4$, 0,5mmol/L $NH_4H_2PO_4$, 2,5mmol/L KCL, 2mmol/L NaCl, 0,2µmol/L $CuSO_4$, 1µmol/L $ZnSO_4$, 0,1mmol/L EDTA-FeNa, 0,02mmol/LH_3BO_3, 5nmol/L $(NH_4)_6Mo_7O_{24}$, and 1 µmol/L $MnSO_4$. Finally, the seedlings were harvested. Then, one part was used to measure morphological indexes and Cd content, and the other part was quickly isolated and frozen in liquid nitrogen, stored at -80 °C, and was used to measure physiological indicators and the analysis transcriptome. All the treatments were replicated three times.

Identification of the growth index and concentration of Cd and other elements. For showing and evaluating Cd treatment results, the plants were photographed by a digital camera and imaged by a scanner. The height and fresh weight were measured. The roots and shoots of the seedlings were oven-dried at 80°C until constant weight and then weighed for dry weight.

For measuring the content of the elements of Cd, Lithium (Li), nickel (Ni), and strontium (Sr), the roots of seedlings were rinsed with deionized water at least three times to remove surface ions, and then, roots and shoots were harvested separately. The samples were dried at 105 °C for 30 min, and then at 80 °C in an oven until completely dry. Then the dry samples were ground into powders. The dry powder of each sample was digested in 5ml HNO_3 overnight (at least for three 3h) at room temperature, then with adding 2ml H_2O_2, continued digesting for approximately 3h at 180 °C. The digested solution was volumized to 25 mL and then studied by an atomic absorption spectrophotometer (ICP-OES, Optima 2100DV, Perkin Elmer).

Study of the antioxidant systems and the ascorbate-glutathione (AsA-GSH) cycle. To further examine and compare the physiological characteristics of Cd tolerance of 62\3 and JB231AC, the concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the leaves of each fresh sample was measured. Antioxidant enzyme systems were evaluated by detecting the activities of superoxide dismutase (SOD) and peroxidase (POD). The AsA-GSH cycle was investigated by detecting the activity of glutathione S-transferase (GST) and the concentration of AsA. The examinations of all the physiological indexes were carried out with the corresponding assay kits (Jiancheng Biotechnology, Nanjing, China).

Statistical analysis. A transfer factor (TF) is the ratio of the concentration of Cd in the shoot to that in the root. The data were statistically analyzed using Excel or GraphPad Prism 8. By GraphPad Prism 8, two-way ANOVA was performed on data sets, with the mean and SD of each treatment calculated. Multiple comparisons with Sidak's test were mainly used to compare the mean values between the treatments ($p<0,05$).

The transcriptome sequencing sample collection and preparation. Plant material acquisition and screening conditions are the same as in 4.2.1. Based on the phenotypic results of 62\3 and JB231AC under Cd gradient and previous studies, treatments of Cd free and 50μM $CdCl_2 \cdot 2,5H_2O$ of the two varieties were selected for further transcriptomic analysis. Total RNAs were extracted from the leaves for analysis of sequencing libraries by NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA).

The library preparation for the transcriptome sequencing. A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext®Ultra™ RNA Library Prep Kit for Illumina®(NEB, USA) following the manufacturer's recommendations, and index codes were added to attribute the sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. The fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer （5X. First-strand cDNA was synthesized using a random hexamer primer and M-MuLV Reverse Transcriptase. Second-strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3’ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure was ligated to prepare for hybridization. To select cDNA fragments of preferentially 240 bp in length, the library fragments were purified with the AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min followed by 5 min at 95°C before PCR. Then PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers, and Index (X) Primer. Finally, PCR products were purified (AMPure XP system), and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Clustering and sequencing. The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumia) following the manufacturer’s instructions. After the cluster generation, the library preparations were sequenced on an Illumina Hiseq 2000 platform and paired-end reads were generated.

Quality control. Raw data (raw reads) of fastq format were first processed through in-house perl scripts. In this step, clean data (clean reads) were obtained by removing reads containing adapter, reads containing ploy-N, and low-quality reads from raw data. At the same time, Q20, Q30, GC-content, and the sequence duplication level of the clean data were calculated. Everything was done by the downstream analyses.

The gene functional annotation. The gene function was annotated based on the following databases: NR (NCBI non-redundant protein sequences), Pfam (Protein family), KOG/COG/eggNOG (Clusters of Orthologous Groups of proteins), Swiss-Prot (A manually annotated and reviewed protein sequence database), KEGG (Kyoto Encyclopedia of Genes and Genomes), and GO (Gene Ontology).

The quantification of gene expression levels. Gene expression levels were estimated by RSEM (Li et al, 2011) for each sample:

1) Clean data were mapped back onto the assembled transcriptome;

2) Read count for each gene was obtained from the mapping results.

The differential expression analysis. The differential expression analysis of two conditions/groups was performed using the DESeq R package (1.10.1). DESeq provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value <0,05 found by DESeq were assigned as differentially expressed.

The GO enrichment analysis. The Gene Ontology (GO) enrichment analysis of the differentially expressed genes (DEGs) was implemented by the top GO R packages-based Kolmogorov–Smirnov test.

The KEGG pathway enrichment analysis. The KEGG (Kanehisa et al., 2008) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism, and the ecosystem from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies (http://www.genome.jp/kegg/). We used KOBAS (Mao et al., 2005) software to test the statistical enrichment of the differential expression genes in KEGG pathways.

2.3 Soil and weather conditions

Soil conditions. The study of the varieties and hybrids collection of sunflowers was carried out in the experimental field of Sumy NAU. The soil of the experimental site is typical for the northeastern Forest-steppe of Ukraine. It is classified as chernozem (black soil) powerful heavy-loam medium-humus on loess-like loam.

According to the agrochemical analysis made in 2019, the soil was characterized by the following indicators: the content of humus in the arable layer (according to IV Tyurin) is 4,0%, the reaction of the soil solution is close to neutral (pH 6,3), the content of easily hydrolyzed nitrogen (according to I V. Tyurin) is 8,3 mg, mobile phosphorus and exchangeable potassium (according to F. Chirikov) are, respectively, 12 mg and 7,2 mg per 100 soils. For each year of the research, the predecessor was spring barley. The main tillage is improved by chilling with plowing to a depth of 22-24 cm. Mineral fertilizers were applied in spring under pre-sowing cultivation in the form of nitroammophos $N_{15}P_{15}K_{15}$ with a rate of 200 kg of fertilizers/ha.

Weather conditions. According to long-term observations, the average annual temperature in the region is 7,4°C and the annual rainfall is 593 mm. The greatest amount of precipitation falls is fixed in the summer and autumn periods. The end and restoration of vegetation of winter crops (transition through the mark of +5°C) occur in the 3-d decade of October and the 2-nd decade of April. The vegetation period of spring crops is from the third decade of April to the third decade of September.

The dynamics of soil and air temperatures assist in sunflowers growing from May to September. The average long-term indicators of the dynamics of monthly temperatures and precipitation of this period are presented in figure 2.1.

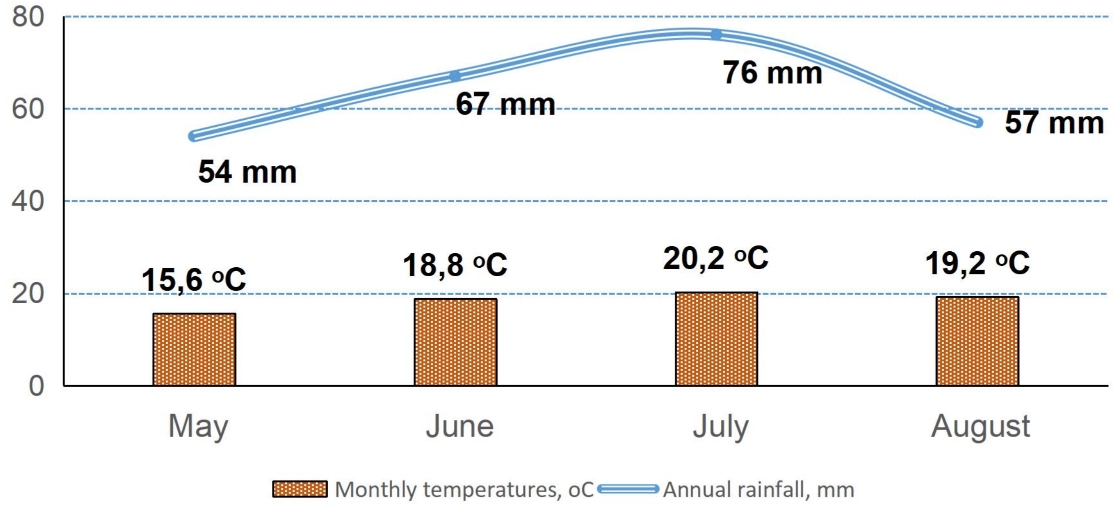

Fig. 2.1 Long-term average values of monthly temperatures and precipitation (meteorological station of the Institute of SGPS NAAS)

According to the research program, field experiments were performed from 2019 to 2021. These years were characterized by certain deviations from the average long-term indicators (Figure 2.2).

Soil and weather conditions in 2019. Significant precipitation and slow melting of snow in the early spring months of 2019 provided the conditions for the full recovery of soil moisture. Higher-than-average values of average daily temperatures in May and June (+15 and +30%, respectively) provided rapid growth and formation of the leaf surface of plants. Under these conditions, the beginning of flowering and the formation of maximum leaf surface area was observed in the first and second decades of June.

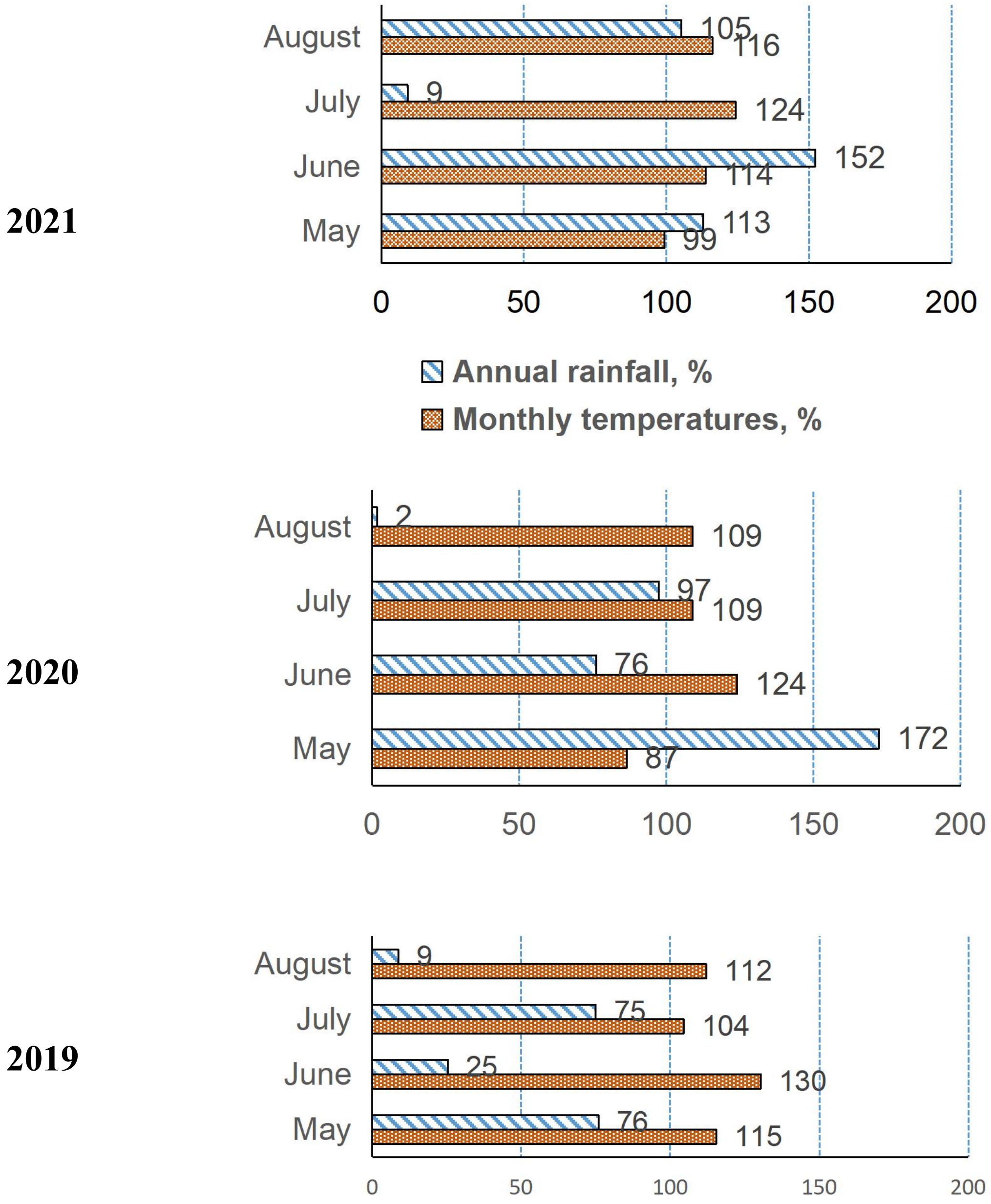

Fig. 2.2. Average monthly temperatures and precipitation in the percent to long-term average indicators (meteorological station of the Institute of SGPS NAAS)

The second half of the growing season (flowering phases, seed filling, and ripening) took place in terms of temperatures above the average long-term values of daily and persistent moisture deficiency. The monthly precipitation in July-August was only 75 and 9% of the normative values. The monthly precipitation in July-August was only 75 and 9% of the normative values. This led to the accelerated rates of generative phases of development in ultra-early and early maturing sunflower genotypes, in which the root system did not provide a sufficient level of water transportation (loss of turgor in the afternoon, intensive death of lower and middle tiers of leaves in the seed filling phase).

On the contrary, genotypes with a vegetation duration of more than 110 days retained the basic indicators of the phases of the embryonic and post-embryonic periods of development. A total of 120 mm of precipitation fell during the growing season, the value of the sum of temperatures was 2614 °C. The value of the hydrothermal coefficient was 0,46. Thus, the course of weather conditions during the growing season in 2019 met the requirements of the analytical background to identify genotypes with intensive development of the root system and its ability to effectively use soil moisture.

Soil and weather conditions in 2020. Weather conditions in the spring of 2020 were characterized by early dates of the beginning of soil thawing, namely on February 18 at average long-term values – on April 5, which provided an opportunity to restore soil moisture reserves due to precipitation in February and April. However, significantly lower than average long-term air temperatures in April and May caused mainly late sowing of sunflowers, delayed emergence of shoots, and low intensity of growth processes in the juvenile phases of plant development. The intensity of growth processes was limited to low night temperatures and prolonged spring frosts.

Thus, the period of sowing, germination, and juvenile phases of sunflower plant development took place under conditions of significantly lower than the average long-term air and soil temperatures. Thus, the average monthly temperature in May was only 87% of the normative value. Under these conditions, the optimal temperature for spring development of the sunflower root system (> +14 °C) was observed only in the first decade of June, or 20 days later than the long-term average. At the same time, such dynamics of temperatures were combined with enough precipitation in May (172% of the long-term average) and close to the normative indicators of the amount of precipitation in June and July.

Such weather conditions caused a shift in the flowering phase of early-maturing sunflower genotypes in the 2nd and 3rd decades of July. In medium-ripe specimens, the formation of the excess leaf surface of plants and the manifestation of the effect of “pulling on light” was observed. The total amount of precipitation of the sunflower growing season in 2020 was 219 mm, and the sum of temperatures is 2447 °C. The value of the hydrothermal coefficient in May, as well as in the summer months was 0,89.

Thus, the main limiting factor in the formation of sunflower yields in 2020 were weather conditions during the zygote formation and seed filling. Embryonic and post-embryonic periods of seed development in some genotypes were shortened, which led to a change in the relationship between the individual parts of the fetus. In general, the combination of weather and climatic conditions was favorable for the potential realization of the genotypes capable of intensive growth at low temperatures in the juvenile phases of development and high attractiveness of the inflorescence in the second half of the growing season.

Soil and weather conditions 2021. The dynamics of weather conditions in the early spring of 2021 were similar to the previous 2020 one. Lower than average long-term air temperatures in April and May were mainly due to the late sowing of sunflowers, delayed emergence of shoots, and low intensity of growth processes in the juvenile phases of plant development. The development of growth processes was limited to low night temperatures. As in the previous year, this was accompanied by a shift in the timing of flowering, the formation of the excess leaf surface of plants, and the manifestation of the effect of “pulling on light”. Later, under the conditions of high temperatures and moisture deficit of the second half of the growing season, the lower unproductive tiers of leaves died out, which, in turn, led to an imbalance in providing the root system with photosynthesis products and reducing its water absorption capacity.

Under these conditions, the embryonic and post-embryonic periods of seed development were shortened, which led to a change in the relationship between the individual parts of the fetus. The result was an increase in the proportion of unfilled seeds and an increase in seed husks. The total rainfall of the sunflower growing season in 2021 was the highest for the research period and amounted to 219 mm, the sum of temperatures of 2592 ^{o}C. The value of the hydrothermal coefficient for four months of the growing season was 0,9.

In general, the weather conditions of the growing season contributed to the identification of genotypes adapted to the temperature and water regimes of the study area, namely the ability to grow intensively in low temperatures and sufficient water supply in the juvenile phases of development with the ability to save moisture in the second half.

During the study period, the average yield of sunflowers in the study area was 3,32, 3,22, and 3,01 t/ha in 2019, 2020, and 2021, respectively. The analysis of weather conditions and indicators of average yield indicates that at this stage the main limiting factor of yield is the low level of heat supply in the spring and the lack of moisture in the second half of the growing season.

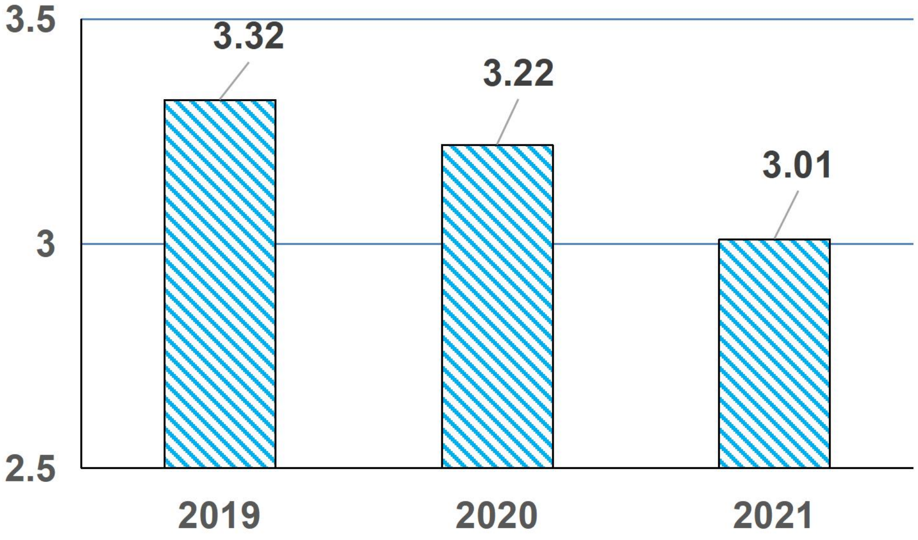

Fig. 2.3 Sunflower yield in the region, t/ha

The results of weather conditions analysis for the growing season of 2019-2021 indicate the absence of abnormal factors. Deviations in temperature and precipitation were not critical for the development of sunflower plants, which allows the use and comparison of research results with sunflower culture in similar soil and climatic conditions.

Conclusions to Chapter 2

The size of the varieties collection of *Helyanthus annuus* L. is sufficient for evaluating the variability of modern sunflower crops in terms of resistance to cadmium accumulation.

The methods of planning, carrying out and collecting digital material of vegetation and field studies, as well as schemes of crossing and testing of hybrid generations correspond to the main current provisions adopted in agronomy.

Testing the selection material involves the use of modern methods and the methods of physiological and genetic research in terms of certified laboratories using industrial devices, equipment, and reagents.

The weather conditions of the 2019-2021 growing season, based on the indicators of total precipitation, and total and monthly temperature dynamics, were close to the average multi-year values and were characterized by some tendency towards warming and aridization. According to the type of soil, its structure, density, and mechanical composition, the soil conditions are typical for the Forest-steppe zone. According to the level of acidity and the content of the main nutrients in the arable layer, the conditions correspond to the average level of recreational load. The content of cadmium in the arable layer corresponds to the indicators of the natural background and is within the limits determined for agricultural land.

Overall, the indicators of the volume of the species collection, the conditions of vegetation, field, and laboratory research, as well as the used set of devices, methods, and statistical tools correspond to the current standards for selection and biological research in agronomy. The obtained results can be reproduced under similar soil and climatic conditions, as well as under laboratory conditions when applying the above-mentioned methods.

CHAPTER 3

SCREENING OF COLLECTIONS AND CREATION OF SOURCE MATERIAL OF SUNFLOWER RESISTANT TO CADMIUM ACCUMULATION

3.1 The collection structure by origin

The program for collection studying in field conditions provided for the assessment of morpho parameter complex and plant growth dynamics during 2019-2021. The study consisted of two stages:

- Indirect assessment of cultivating sunflower samples for the resistance to cadmium accumulation in terms of vegetation experiment (2019);
- Assessment of the collection according to the complex breeding and valuable traits in conditions of field experiment (2019-2021).

104 samples were analyzed at the first stage of the research. However, in the second stage, parts of the samples were excluded from the work due to the insufficient amount of seed material and low seed germination. Thus, a full set of studies was conducted for 95 selection samples (Fig. 3.1).

Most of these samples, namely, 36 and 25 (38 and 26%, respectively) were provided by the National Center for Plant Genetic Resources of Ukraine and Sumy National Agrarian University. A smaller part of samples, namely, 19 and 8 number (20 and 9%) were provided by the Institute of Oil Crops and the Institute of Agriculture of the Northeast of Ukraine. A significant part of the collection, namely 7% (7 samples), was made up of commercial varieties and hybrids, included in the State Register, and distributed in production of the North-Eastern Forest Steppe of Ukraine.

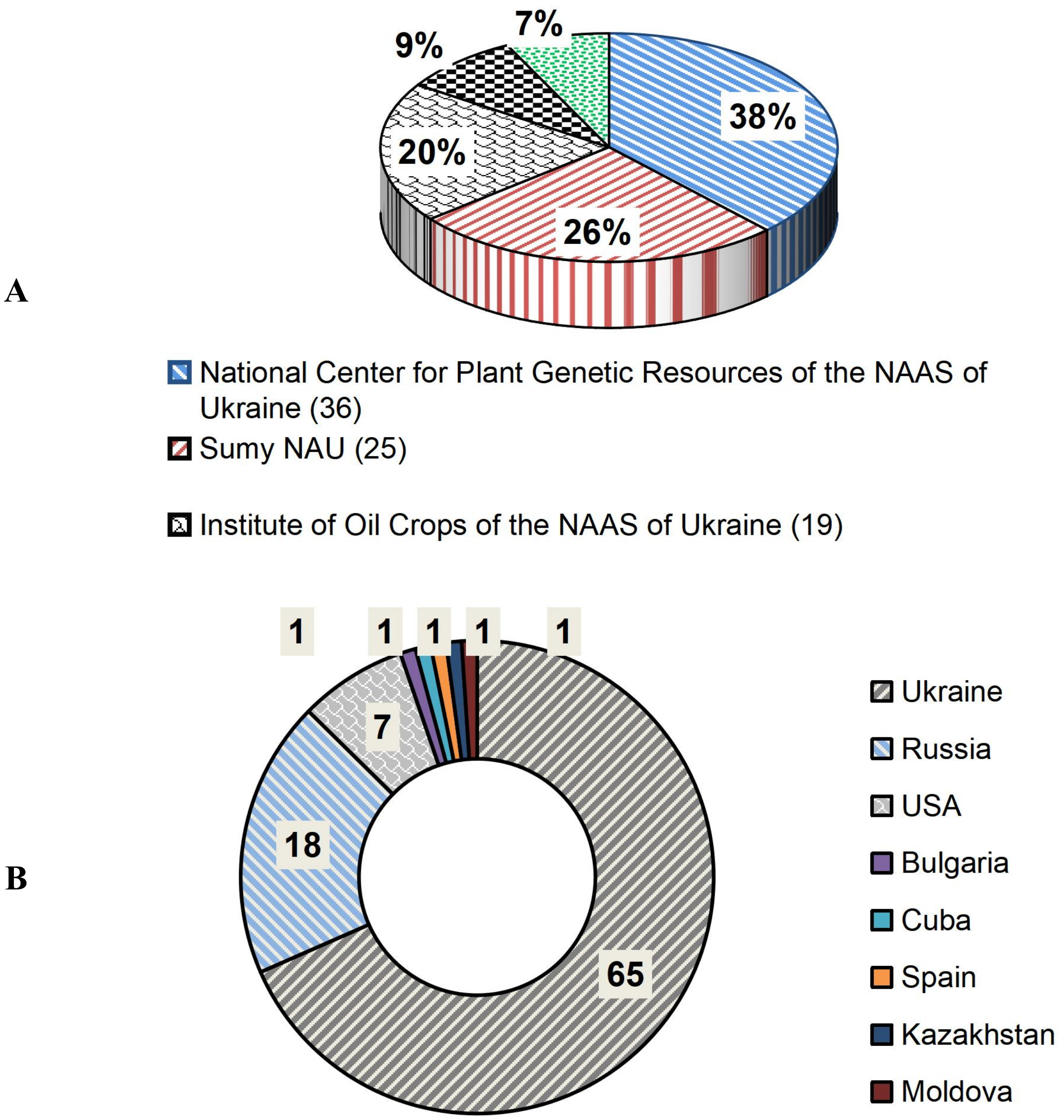

Fig. 3.1. The structure of the basic collection of selected samples of sunflower A – by institutions; B – by geographical origin.

By geographical location, the collected samples was represented by 8 countries (Fig. 3.1b). The largest number – 65 were developed in Ukraine. Eighteen and seven samples were represented by the selection centers of Europe and the USA, respectively. Bulgaria, Cuba, Spain, Kazakhstan, and Moldova presented per one selection sample.

3.2 Evaluation of the resistance to cadmium uptake in vegetation experiment condition

Soil is the basis of human survival and development and is one of the indispensable natural resources in the global ecosystem. It is closely related to agricultural production, the environment, and sustainable human development. Due to human activities and the unreasonable use of pesticides and fertilizers, cadmium pollution has become increasingly serious in recent years (Dias et al., 2013; Zhang et al., 2015b). Cadmium is one of the most toxic heavy metals. Once the exogenous Cd enters the soil, it can greatly affects the growth and development of plants. It will gradually accumulate and harm crops for a long time and endanger human health through the transmission of the food chain leading to the decline of crop yield capacity and quality. To make matters worse, cadmium can accumulate in the human body through the food chain and induce many diseases affecting human health (Dias et al., 2012).

Previous studies have shown that phosphate fertilizers often contain high amounts of heavy metals such as Cd. Thus, long-term application of phosphate fertilizers will inevitably lead to excessive Cd content in soil (Bolan et al., 2014). Song et al. (Song et al., 2013) collected and analyzed the heavy metal database of cultivated soil in 138 typical regions of China, and the results showed that 16,66% of cultivated land was polluted by heavy metals. The probability of Cd pollution in cultivated land soil was 25,20%, far higher than other heavy metal elements. Zhang et al. (Zhang et al., 2015c) collected 486 studies on the content of Cd in China's cultivated soil. The analysis showed that the average content of Cd in China's cultivated soil was 0,27 mg/kg. Some studies also pointed out that Cd in the soil of farmland in China increased at an average rate of 0,004 mg/ kg much higher than that in the soil of Europe (0,000 33 mg/kg) (Lei et al., 2009).

The crops grown worldwide are mainly divided into ten categories: fruit and vegetables (including citrus, grape, potato, and pear), grains (mainly wheat), corn, soybean, rice, rape, cotton, sugar beet, and sunflower. Although the sunflower planting area is small, because of its high edible, medicinal, and economic value, it has attracted much attention. Sunflower oil is rich in unsaturated fatty acids, low in fat, and can inhibit the synthesis of cholesterol in the human body. Currently, sunflower oil is the preferred edible oil in developed countries. Sunflower seeds contain high protein, edible fiber, plant sterols, phospholipids, and linoleic acid, which can effectively prevent heart disease, hypertension, arteriosclerosis, and other diseases, as well as help to reduce the blood cholesterol level of the human body and improve human immunity (Zhao et al., 2011). Sunflower is mainly planted in temperate and subtropical regions competing with soybean, rape, palm, and other vegetable oil crops. Sunflowers can be divided into oil type and edible type. Most of the sunflower varieties planted worldwide are oil-type.

According to statistics, the global sunflower planting area in 2015 was about 25,4 million hm^2, up 1,0% year-on-year. The planting area of Russia ranks the highest in the world, with 7 million hm^2, accounting for 27,6% of the total planting area of the world, with a year-on-year increase of 2,6%. The planting area of Ukraine is 5,1 million hm^2, ranking second in the world, with a year-on-year decrease of 3,8%. The planting area of Argentina is 1,47 million hm^2, ranking third, with a year-on-year increase of 12,7%. China ranks fourth with 920,000 hm^2, with a 3,2% year-on-year increase. It is followed successively by the United States (750,000 hm^2), Spain (740,000 hm^2), Hungary (630,000 hm^2), France (610,000 hm^2), South Africa (580,000 hm^2), and India (500,000 hm^2). As the per unit output, Ukrainian sunflower (2,16 tons /hm^2) was significantly higher than that of Russia (1,39 tons /hm^2). The total output of Ukrainian sunflowers is higher than that of Russia (11 million tons) (Zhang & Gu, 2018).

Cadmium accumulation varies greatly among different plant varieties due to their different genotypes. It is generally believed that the cadmium accumulation of *Crucifera* crops (such as mustard, rape, and radish) is higher than that of *Gramineae* (such as rice, wheat, and maize), while the cadmium accumulation of leguminous crops (such as soybean and pea) is lower (Arthur et al., 2000). The accumulation of cadmium in the same variety was significantly different with different genotypes. Michalik (1995) studied the differences in cadmium accumulation among four carrot varieties and found that cadmium accumulation characteristics of different carrot varieties were very different. The difference in cadmium accumulation between rice and hybrid rice has been studied. The result showed that hybrid rice had a stronger ability to accumulate cadmium than conventional rice. Subsequently, genotypic differences in cadmium accumulation were also found in many other crops.

Soil cadmium pollution is a long-standing problem. It is of great significance to study the cadmium tolerance mechanism of crops and the cultivation of low cadmium materials. Currently, studies have been carried out on the cadmium tolerance mechanism of various crops. Sunflower is an important economic crop in Ukraine. According to EU standards, sunflower cadmium accumulation should not be more than 0,05 mg/kg. Facing the increasingly severe problem of improving the soil polluted by cadmium, cultivation of sunflower varieties with low cadmium accumulation can effectively reduce the cadmium content in seeds, fundamentally solving the problem of cadmium entering the body through the plant feeders such as sunflowers. Therefore, this study carried out the screening of sunflower varieties with low cadmium content.

In our experiment, 104 sunflower varieties were used as test materials, and the above-ground biomass and cadmium content were used in hydroponics and pot experiments to screen out sunflower varieties with the significant difference in cadmium accumulation and provide experimental materials for studying the mechanism of cadmium accumulation. (Fu et al., 2019)

3.2.1 Growth index of sunflower samples

It was established that the final phase of vegetative mass growth of sunflower plants (more than 90 %) from the final values of the index (termination of vegetation) in terms of vegetation experiment was the beginning of the flowering phase. The duration of the “seedlings-flowering” period varied from 55 to 73 days (Fig. 3.2).

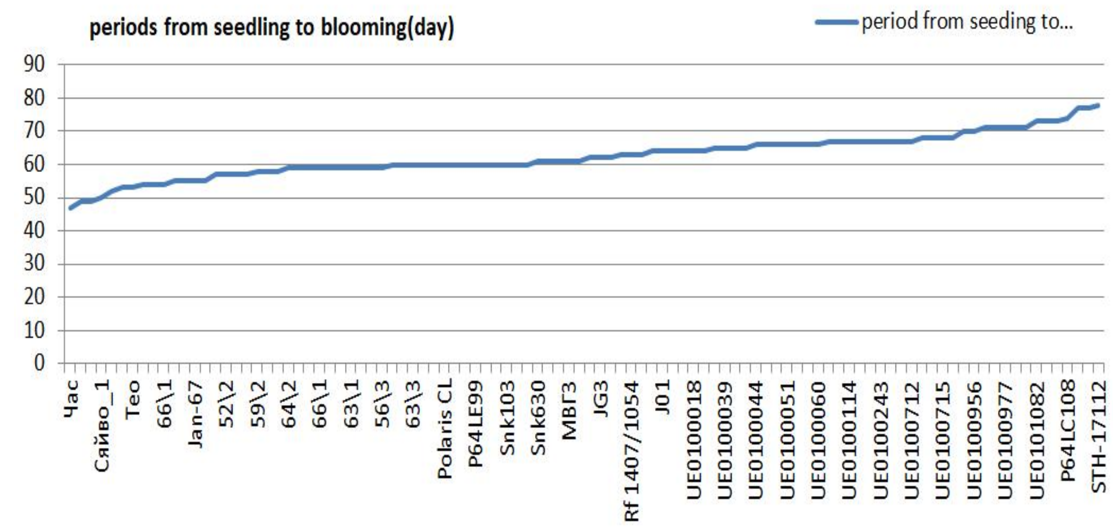

Fig. 3.2. Periods from seedling to flowering, 2019.

The range of plant mass indices in the experiment ranged from 1,3 to 2,9 g/plant (Fig. 3.3). The values range of the cadmium content indicator in the above-ground part varied from 0,66 to 2,62 mg/kg. The average content of cadmium in the above-ground part of the plants was 1,42.

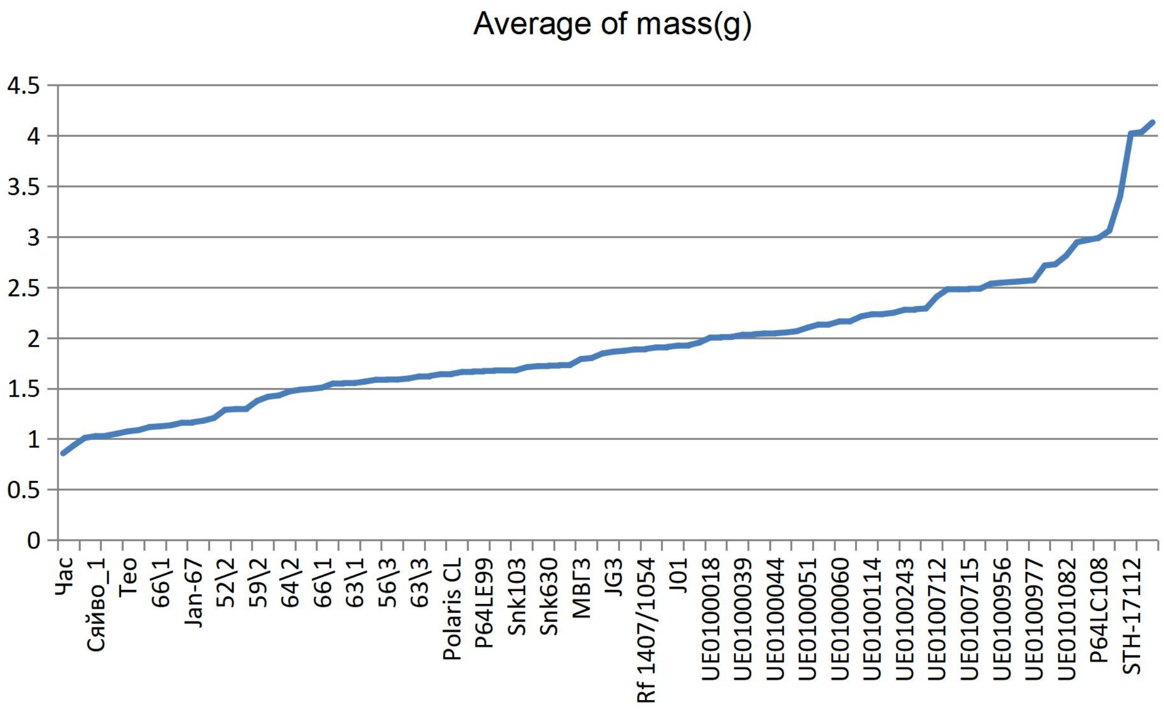

Fig. 3.3. Average of plant mass, 2019.

3.2.2 Differentiation of the sunflower collection according to the indicator of resistance to cadmium accumulation

The idea proposed by Yin-Ming (Yin-Ming et al., 1997) regarding the possibility of assessing the resistance of sunflower plants to cadmium accumulation based on the results of their testing in terms of a vegetation experiment was the basis of the method we developed for the indirect assessment of selection material of sunflower in analyzing background condition .

The parameters of such background were determined experimentally as the minimum value of cadmium concentration in the soil that provides a clear, statistically significant, intervarietal difference in the rates of cadmium absorption in the vegetative organs of sunflower and amounted to 1,0 mg/kg . Later, the obtained data were compared with the results of field experiments.

In general, the use of an analytical background with a cadmium concentration of 1,0 mg/kg and the cultivation of selected samples under controlled environment conditions until the R5 phase allowed us to establish that the range of values of the cadmium concentration indicator in the aerial part of plants varied from 0,5 to 2,6 mg/kg. Within the defined range, the collection was differentiated into 5 groups, (Fig. 3.4).

The group of plants with high resistance to cadmium accumulation in the aboveground phytomass included 11 samples with a value of the indicator from 0,5 to 0,9 mg/kg: S2K670, JB231AC, UE0100938, IPS\125, 64\3, Polaris CL, Reason, 56\3, Snk630, UE0100977, and UE0100018. The share of this group in the total collection was 10,6%, and the average group value of the cadmium content index was 0,76 mg/kg. The most numerous were the groups with a good and satisfactory level of resistance, in which the range of values of the cadmium concentration indicator was 0,91-1,3 and 1,31-1,7 mg/kg, respectively.

The group with a good level of resistance included samples such as Neon_2011, KG49, UE0100114, 54\1, UE0100044, J01, DN47-2, Amis CL, P64LE99, Snk103, 67/1, UE0101177, 69\1, UE0100981, APS49, UE0100939, Haze, RF 1407/1054, UE0100041, DN-47-9, 59\1, UE0100043, 66\1, from Sumy, Z1064, UE0100051, 64\2, Theo, Esman, UE0100035, 71\1, and 62\3. The share of the group in the collection was 32%, with an average group value of 1,12.

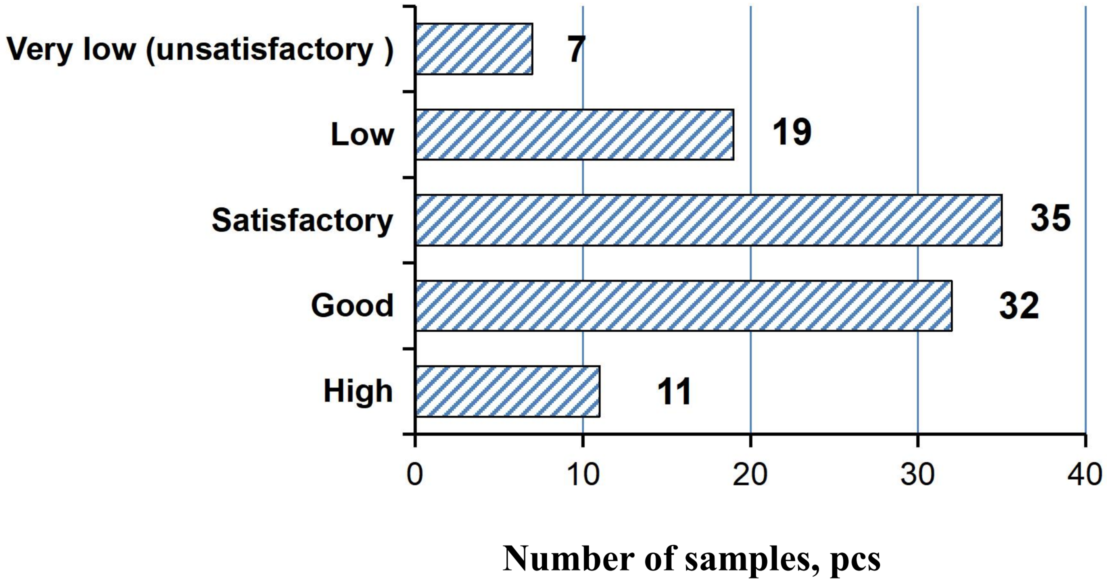

Fig. 3.4. The distribution of the sunflower collection according to the indicator of resistance to the Cd accumulation in the aboveground phytomass. The vegetation experiment of 2019 of analyzing the background of cadmium 1,0 mg/kg.

High – 0,5-0,9 mg/kg; Good – 0,91-1,3 mg/kg; Satisfactory – 1,31-1,7 mg/kg; Low – 1,71-2.1 mg/kg; Unsatisfactory – 2,11-2,5 mg/kg.

The group with a satisfactory level of resistance included 35 samples or 33,7% of the collection, namely, Syavo_1, UE0100039, APS leader, UE0100045, IPS\54, J071/1, UE0100714, UE0100026, UE0100956, The fiddler, MVG2, 52\2, 66\1, UE0100713, Time, M1049, 65\2, UE0101021, Radiance, 55\2, UE0100047, 63\1, UE0100118, UE0100274, IPS\135, 61\1, IPS\235, MVG3, UE0101082, IPS\134, UE0101323, UE0100238, UE0100968, 58\2, and UE0100077. The average value of cadmium content in this group was 1,49 mg/kg.

The group with a low level of cadmium resistance included 19 samples or 18,3% of the total number of the collection. They were UE0100964, IPS\73, 57\1, UE0100711, UE0100243, 59\3, 68\1, 65\1, Onyx_2007, Onyx, IPS\232, 63\3, UE0100084, 57\2, 424924p7-4, 59\2, M1043, UE0100712, and IPS\240. The average value of the indicator for the group was 1,86 g/kg, varying from 1,71 to 2,1 mg/kg.

The group with an extremely low level of resistance had the smallest number of samples. The group included 7 selected samples with a cadmium content of more than 2,11 mg/kg, namely, PR63LL01, UE0100056, UE0100715, 62\3, UE0100052, JG3, and UE0100060. The share of this group in the collection was 6,7%.

Thus, according to the results of the vegetation experiment with the analyzing background, the range of values of the cadmium concentration indicator in the aboveground phytomass of sunflower plants was determined, which was 0,5–2,26 mg/kg. The results of the experiment provided the possibility of preliminary differentiation of the sunflower collection with the selection of groups with different level of resistance to cadmium accumulation.

3.3 Assessment of resistance to cadmium accumulation in field experiment condition

The study of the collection in field experiment condition was carried out in 2019-2021. Due to the limited number of seeds or their low germination, 9 selection samples were excluded from the research. They were Onyx_2007,(Sumy), 68\1, 62\3, Z1064, DN47-2, UE0100039, UE0100043, and UE0100711. Thus, a complete research program was carried out for 95 selection samples.

The main determining feature of the collection study in field experiment condition with a natural background of cadmium (0,21 mg/kg) was the resistance of plants to the accumulation of the metal in the seeds. According to the results of a 3-year experiment, the range of the cadmium content index varied from 0,23 to 0,43. The average value of the cadmium content index in seeds from the collection was 0,34±0,01 mg/kg. The value of the variation coefficient was 12,12% (Fig. 3.5)

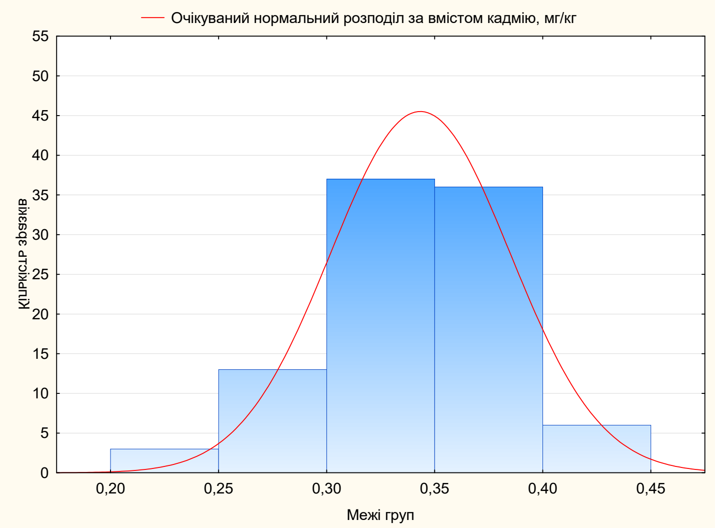

Fig. 3.5. The distribution of the sunflower collection by the indicator of cadmium content in seeds, 2019-2021.

The first and second groups with the minimum level of cadmium accumulation in seeds included 16 samples (3+13), which together amounted to 16,8%. Mostly, these were SNAU samples: 64\3, 56\3, Neon-2011 and samples provided by the National Center of Plant Genetic Resources of Ukraine – UE0100938, UE0100018, UE0100977, IOK – S2K670, JB231AS, Snk630, and some commercial varieties and hybrids distributed in the region such as Sumico, Polaris CL, and Rezon.

The groups with cadmium concentration levels from 0,31 to 0,35 and from 0,36 to 0,40 mg/kg, 37 and 36 samples, or 39 and 38%, were the most numerous. The highest frequency in the value close to the average for the collection of 0,34±0,01 indicates the relative homogeneity of modern sunflower crops in terms of resistance to cadmium accumulation.

The number of the group with the maximum value indicator of cadmium accumulation in seeds was quite high (> 0,4 mg/kg). This group (which share was 6,3%) included 4 samples provided by the National Center of Plant Genetic Resources of Ukraine – UE0100056, UE0100715, UE0100052, UE0100060, and one sample from the collections of SNAU – 62\3, and IOK – JG3.

Data comparison of the vegetation and field experiment on the indicators of the cadmium content in the seeds showed the similarity of the results regarding the differentiation of the collection by the level of resistance to the accumulation of cadmium. Selected ampless of 63,8% analyzed by the method of indirect assessment corresponded to the indicators obtained in the field experiment

The highest rates of compliance were in the groups with unsatisfactory and low levels of resistance – 85,7 and 84,2%, respectively. A group with a high level of resistance was also characterized by a high share of joint (common) hybrids of 76,1%. In all cases of group inconsistency, samples were transferred to the next (higher- or lower-ranked) group. This indicates that the reduced proportion of shared samples in the groups with good and satisfactory resistance levels of 40,6 and 42,8% can be explained by the mismatch of the boundaries of the selected groups.

Table 3.1

Comparative characteristics of the results of vegetation and field experiments on the evaluation of selection sunflower samples for resistance to cadmium accumulation

The group with resistance to Cd	Field experiment, 2019 -2021	Vegetation experiment (2019)		
	samples in the group, pcs	samples in the group, pcs		part of samples in the group, field experiment, %
		total	including common ones	
High	8	11	8	72,7
Good	17	32	13	40,6
Satisfactory	28	35	15	42,8
Low	35	19	16	84,2
Unsatisfactory	7	7	6	85,7

The high proportion of common (joint) hybrids (more than 2/3) in groups with low and unsatisfactory resistance indirectly indicates the universality of genetic mechanisms of cadmium accumulation in vegetative organs and sunflower seeds. On the contrary, the decrease in the share of common hybrids in groups with high and good levels of resistance indirectly indicates a difference in the control mechanisms of cadmium accumulation processes in vegetative organs and seeds.

Therefore, the method of indirect evaluation provides high efficiency of negative selection against the trait and can be used for the preliminary selection of samples with a high level of resistance to cadmium accumulation in seeds.

3.3.1 The collection differentiation by the complex of economic and valuable characteristics

Modern sunflower crop is based on single-head forms of annual sunflower. At the same time, the multi-head form is widely used (mainly as a parent component) in the selection, as well as in heterosis seeding. The presence in the collection of various forms of selection samples complicated the procedure of their evaluation by indicators of vegetative development and elements of plant productivity. The unified indicator for different forms of sunflowers is the duration of plant vegetation. The most convenient parameter that makes it possible to differentiate samples with a difference of one day or more is the indicator of the length of the “seedling-flowering” period. Fig. 3.6 shows the range of indicator values, the boundaries of selected groups, and their representation by selection samples.

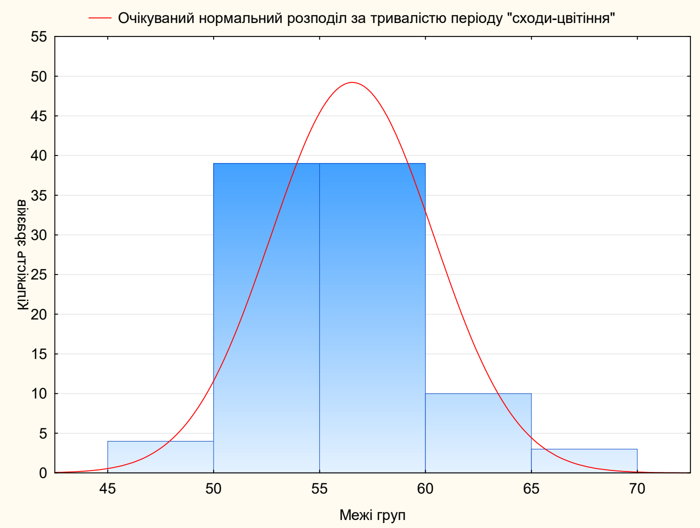

Fig. 3.6. The distribution of the sunflower collection by the length of the “seedling-flowering” period, 2019-2021.

The mean and standard error for the collection was 56,55 ± 0,40 days. The indicator range varied from 50 to 66 days. The value of the coefficient of variation was 6,81. In general, a frequency distribution close to normal was provided by the selection of 5 groups with an intragroup interval of 5 days.

The minimum number of samples was 4,2 and 3,1% in the groups of ultra-ripe and medium-ripe forms, respectively. The group of ultra-early samples with a length of flowering period less than 50 days was represented by three selection samples of the Sumy National Agrarian University, such as Esman, Chas, Onyx, and the sample of UE0100052. The characteristic features of plant vegetative development in this group were their short stature and a small number of leaves – 14-19, with an average value for the collection of 22,1 leaves/plant. The group of late-ripening samples (> 65 days) was represented by the samples UE0100114, UE0100714, and UE0101021. The samples had indicators of the number of leaves of 38-44 pcs/plant, the maximum for the collection, and had values of the indicator of the area of one leaf higher than the average for the collection, as well as the ability to form and maintain high indicators of LAI (index of leaf area) – 5,45-6,64 with an average for collections of 3,07 m^2/m^2.

The most numerous samples, covering total of 82% of the collection, were the group with the length of the “seedling-flowering” period in the range from 51 to 55 days and 56 to 60 days, 39 samples or 41,05% in each group. According to most indicators of vegetative development, the plants of these samples had values close to the average for the collection. The largest number of samples with the minimum level of cadmium accumulation – 6 and 5 – belonged to the groups with the duration of the “seedling-flowering” period of 51-55 and 56-60 days, respectively. However, the largest number of samples resistant to cadmium accumulation in percentage (more than 30% each) was represented in the groups of late-ripening genotypes with a length period of more than 60 days.

An important indicator of the vegetative development of plants, which correlates with indicators of plant productivity and crop yield, is the area of the leaf surface of individual plants and the ability of the crop to form and maintain high indicators of the leaf surface index for a long time. The informativeness of the last indicator is determined by the peculiarity of the weather and climate conditions of the research area, namely, the level of moisture is higher than in traditional areas of sunflower cultivation, with lower indicators of the temperature sum during the growing season. Such conditions provide the possibility of differentiating the collection of samples according to their ability to form high indicators of the leaf surface index. Considering the final sowing density of 55,000 plants/ha adopted in field experiments, a high value of the leaf surface index should be combined with a sufficient level of shade tolerance.

Thus, according to the results of the research (Trotsenko et al.), the critical level (which is accompanied by a change in the architecture of the crop because of the death of the lower and even the middle layer of leaves) is the LAI value of more than 4,0 m^2/m^2. At the same time, according to the author, the low level of adaptability of heat-resistant hybrids of southern origin is explained by low indicators of LAR, which value, as a rule, is in the range of 1,8 – 2,2 m^2/m^2

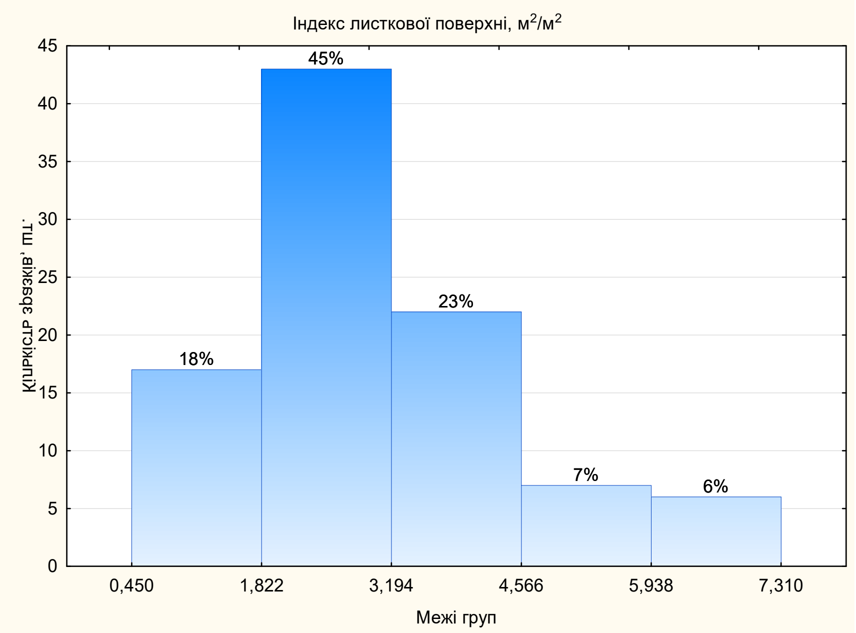

Fig. 3.7. The distribution of the sunflower collection by the leaf area index (LAI), 2019-2021.

Correct, close to the normal distribution of the collection required the selection of 5 groups in the range from 0,45 to 7,3 m^2 with a group interval of 1,37, Fig. 3.7. It is worth noting that this indicator was characterized by one of the highest values of the coefficient of variation – 44,7%. (Fig. 3.7)

Regarding the group distribution of the samples, the second and third group were the most numerous with a range of indicators from 1,83 to 4,5 m^2/m^2. More than 70 samples, or 45 and 23%, respectively, were included in these groups. The groups were dominated by selection samples with a high level of adaptability to the conditions of the research area developed at the Sumy National University, the Institute of Agriculture of Northeast Ukraine of the National Academy of Sciences, and the samples, provided by the National Center of Plant Genetic Resources of Ukraine, and commercial hybrids common in the region.

As in the previous case, in each of the isolated groups with a different percentage of participation, samples with minimum and maximum levels of cadmium accumulation were presented. The exception was the group with LAI more than 5,9, which included sample of UE0100114 with the minimum level of cadmium concentration in seeds.

The selection value of the collection is determined by the range of variability and average values of the main selection control features, primarily plant productivity. For the collection, the mean value and its error was 33,64±0,72, varying in the range from 11,70 to 63,30 with a value of the coefficient of variation of 20,75%. With such indicators, close to normal distribution was provided by the selection of groups with a step of 10,3 g. (Fig. 3.8).

According to the distribution structure, the groups with productivity from 36 to 45 g/plant were the most numerous. This included samples ecologically adapted to the conditions of the zone, provided by SNAU, Institute of Agriculture of Northeast Ukraine of the National Academy of Sciences, and commercial hybrids presented in the zone. However, the maximum performance was noted for samples of UE0101177, UE0100047 UE0100938, and UE0100045 provided by the National Center of Plant Genetic Resources of Ukraine.

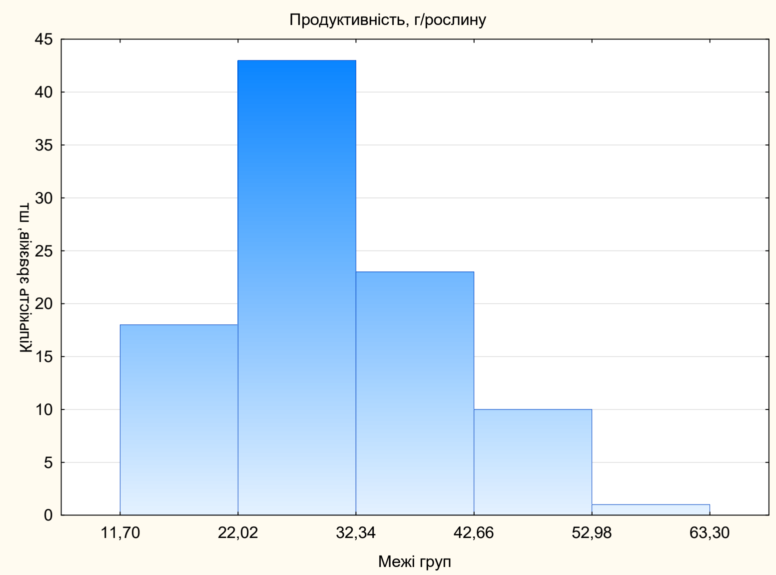

Fig. 3.8. The distribution of the sunflower collection by plant productivity indicator, 2019-2021.

The group characteristics of these samples were the higher values of the leaf density indicators and the area of one leaf than the average for the collection. Regarding the number of samples with different levels of cadmium accumulation, their representation in the main groups (1-4) was relatively proportional. Concurrently, a high level of differentiation of the group of low-productivity samples deserves attention. From a total number of 18 samples with productivity of less than 22,02 g/plant, four samples (or 22%) were characterized by the minimum level of cadmium accumulation, and three samples (or 17%) belonged to the groups with the maximum level of cadmium concentration in seeds.

3.4 The selection of sunflower samples for crossbreeding

One of the tasks of collection study was the selection of samples for crossbreeding to create sunflower source material with a controlled level of cadmium accumulation. In the study, the method of cluster analysis was applied to the group selection samples according to a complex of selection-valuable traits.

The results of the grouping of 16 low-cadmium samples are shown in Fig. 3.9. The analysis indicates the morphological heterogeneity of this group, which made it possible to distinguish three intragroup clusters. Thus, cluster A included the samples of UE0100114, UE01000114, UE0100977, and UE0100018. Samples are characterized as late-ripening ones (length of the “seedling-flowering” phase is > 64 days), high (> 193 cm), and low-yielding. In our opinion, the low attractiveness of inflorescences led to the low intensity of cadmium transfer to seeds. Thus, the inclusion of these hybrids in crossing is impractical, since the low content of cadmium in the seeds is combined with low plant productivity.

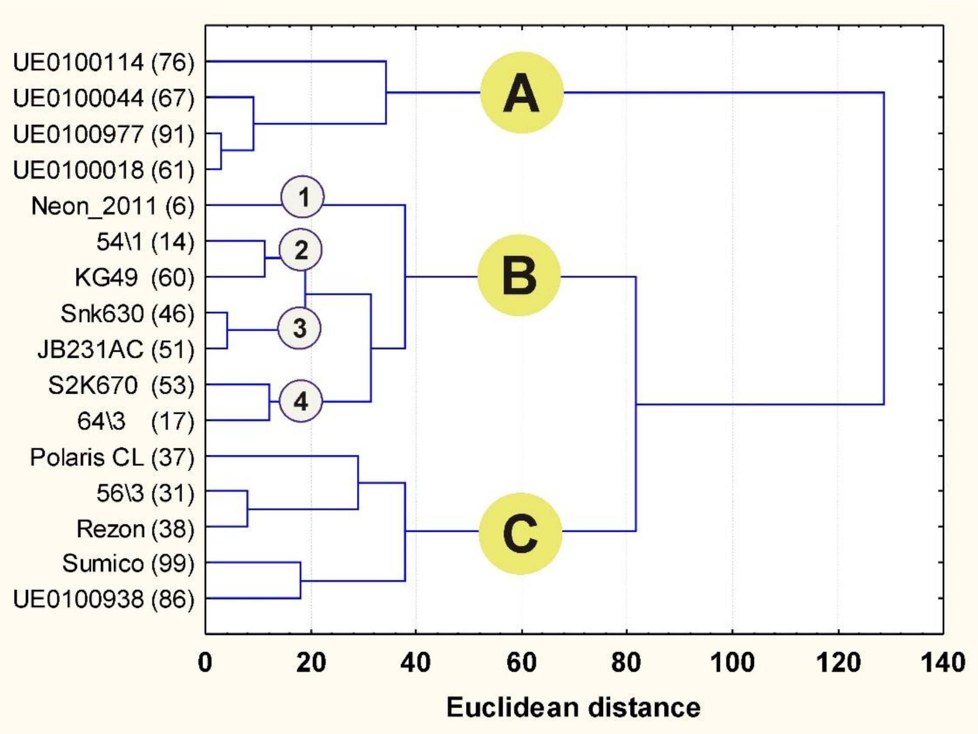

Fig. 3.9. The differentiation of a sunflower samples group with a low level of cadmium accumulation in the seeds, 2019.

Cluster C had the opposite characteristics, which included: the sample provided by the National Center of Plant Genetic Resources of Ukraine – UE0100938, line of 56/3 (SNAU), and three commercial hybrids, such as Sumico, Rezon, and Polaris CL. Common characteristics of these samples were the average level of stem height (143,3-17733 cm), close to the level of plant productivity (22,6-52,0 g/plant), and higher than the average for the collection. In this group, such characteristics indicate the availability of more complex mechanisms for blocking the transportation of cadmium from vegetative organs to seeds.

Cluster B was quite heterogeneous in composition. It included short and semidwarf selection samples with different levels of productivity. Within the cluster, a group of samples was selected. They were Neon_2011, 54\1, and KG49 with a stem height of lower than 1 m , LAI indicator higher than the average values for the collection, and cadmium content in seeds at the level of 0,9-1,0 mg/kg. The second cluster group consisted of samples with indicators of productivity and development, close to the average in the collection, and with minimum indicators of cadmium content in seeds – 0,55-0,75 mg/kg.

The group with the maximum level of cadmium accumulation in the seeds. The structure of the "high cadmium" group of samples was also based on the difference in the total phytomass of the plants and their seed productivity. Within the group, a cluster of samples with a short stem of 95-125 cm and high values of the LAI indicator – 0,7-1,95 – was selected (UE0100712, UE0100052, JG3, and 62\3). In our opinion, a high concentration of cadmium in the seeds could be realized due to the increased attractiveness of the inflorescence under the conditions of a limited number of mineral elements in the above-ground part of the plants.

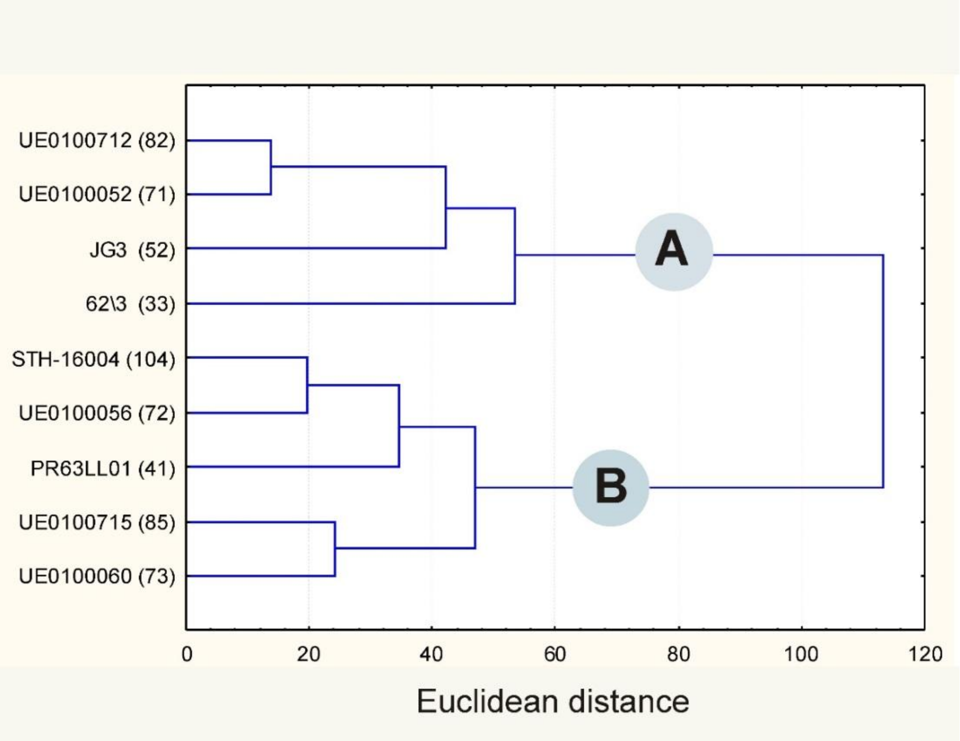

Fig. 3.10. The differentiation of a samples group of sunflowers with a high level of cadmium accumulation in the seeds, 2019.

In contrast, cluster B included tall samples with a stem height of 170-203 cm, such as UE0100060, UE0100715, UE0100056, STH-16004, and PR63LL01. Genotypes of this group were characterized by indicators of the leaf surface area of 0,5-0,8 m^2/plant, which were higher than the average, and plant productivity of 20,6-44,0 g/plant, close to the average for the collection.

The availability of a significant stock of photosynthesis products in samples of this cluster suggests a more passive type of cadmium uptake by seeds.

However, as in the previous case, a necessary condition for the accumulation of cadmium in seeds is the increased concentration and biological activity of this element in vegetative organs. Thus, in both groups, the presence of one unified mechanism of the transportation of biologically active cadmium from vegetative organs to fruits is observed, which implies an increased concentration and sufficiently high biological activity of this element in the vegetative organs of plants.

3.5 Crossing results and characteristics of inter-varietal sunflower hybrids

The heterogeneity of the basic collection of the selected samples and the difference in the mechanisms of ensuring a low level of cadmium accumulation in sunflower seeds was confirmed by the results of inter-varietal crossings.

In total, seeds of 73 hybrid combinations were obtained (in sufficient quantity for F_1 and F_2 testing). Among them, there were 46 hybrids in combinations between samples with minimum cadmium content, 13 hybrids in combinations between samples with maximum cadmium content, and 14 hybrids in intergroup crossings. The calculation of the level of phenotypic dominance of the resistance trait to cadmium accumulation was carried out based on the results of the analysis of the seeds of the 2020 yield (Table. 3.2.)

The following results have been obtained: when crossing “low cadmium” samples, only a fifth or 19% of them inherited the trait of resistance to cadmium accumulation by the type of heterosis. A significant part of the hybrids inherited the trait by the type of partial positive dominance (9,5%) and by intermediate dominance for parental forms (9,5%). However, in most cases, 57,1 and 4,8% of the trait inheritance occurred according to the type of depression and partial negative dominance. In crossing variants in the group of “high cadmium” selection samples and between group crossings, the predominant type of inheritance was also depression and partial negative dominance.

Table 3.2

Inheritance of resistance to cadmium accumulation, %, (F_1, 2020)

Type of inheritance	Scheme of crossing combination		
	мin	мax	мin x мax
Heterosis	19	14,3	33,3
Depression	57,1	85,7	50
Partial positive dominance	9,5	0	0
Partial negative dominance	4,8	0	16,7
Intermediate inheritance	9,5	0	0

In crossing variants in the group of “high cadmium” selection samples and between group crossing, depression and partial negative dominance were also the predominant types of inheritance. The total share of these types of phenotypic inheritance was 85,7% when crossing “high cadmium” samples and 66,7% in intergroup crossings.

The second generation of inter-varietal hybrids was obtained in the process of controlled pollination under insulators of 5-6 plants of each hybrid. The results of the analysis of seeds of the first and second generation of hybrids (2020 and 2021 harvest) are presented in Table. 3.3.

The average value of cadmium content in seeds for the experiment was 0,41 mg/kg with a range of variation from 0,05 to 2,24 mg/kg. The minimum values were noted in hybrids 19/06, 19/32, and 19/67 obtained in combinations of Rezon X PR63LLO1, Zorya X JG3, and JB231AC X X51B, respectively. The maximum value of the indicator was hybrid 19/11 (a combination of the commercial hybrid Polar CL and the Sumy NAU line 64/3).

Table 3.3

Cadmium content in seeds of inter-varietal hybrids (2020-2021)

N of hybrid	Origin		Cd content, mg/g
	collection number	selection sample	
19/01	17 x 31	64/3 x 56/3	0,48
19/02	17 x 38	64/3 x Rezon	0,16
19/03	17 x 86	64/3 x X51Б	0,27
19/04	17 x 90	64/3 x Chk.giant	0,65
19/05	31 x 17	56/3 x 64/3	0,09
19/06	38 x 41	Rezon x PR63LLO1	0,05
19/07	31 x 38	56/3 x Rezon	0,28
19/08	31 x 86	56/3 x X51Б	0,16
19/09	31 x 90	56/3 x Chk.giant	0,36
19/10	31 x 99	56/3 x Sumico	0,1
19/11	37 x 17	Polar CL x 64/3	2,24
19/12	37 x 31	Polar CL x 56/3	0,4
19/13	37 x 86	Polar CL x X51Б	0,18
19/14	37 x 90	Polar CL x Chk.giant	0,37
19/15	37 x 99	Polar CL x Sumico	0,09
19/16	38 x 31	Rezon x 56/3	0,09
19/17	38 x 37	Rezon x Polar CL	0,21
19/18	38 x 53	Rezon x S2K670	0,21
19/19	38 x 86	Rezon x X51Б	0,18
19/20	38 x 99	Rezon x Sumico	0,09
19/21	46 x 37	Snk630 x Polar CL	0,23

19/22	46 x 38	Snk630 x Rezon	0,24
19/23	51 x 17	JB231AC x 64/3	0,44
19/24	51 x 31	JB231AC x 56/3	0,16
19/25	51 x 37	JB231AC x Polar CL	0,66
19/26	51 x 38	JB231AC x Rezon	2,01
19/27	53 x 31	S2K670 x 56/3	0,19
19/28	53 x 37	S2K670 x Polar CL	0,2
19/29	53 x 86	S2K670 x X51Б	0,18
19/30	53 x 90	S2K670 x Chk. giant	0,37
19/31	53 x 99	S2K670 x Sumico	0,1
19/32	72 x 52	Zorya x JG3	0,05
19/32	86 x 61	X51B x Slobozhankyi	0,36
19/33	90 x 31	Chk.giant x 56/3	0,36
19/34	90 x 37	Chk.giant x Polar CL	0,55
19/35	90 x 38	Chk.giant x Rezon	0,38
19/36	90 x 61	Chk.giant x Slobozhankyi	0,47
19/37	99 x 31	Sumico x 56/3	0,43
19/38	99 x 37	Sumico x Polar CL	0,11
19/39	99 x 38	Sumico x Rezon	0,14
19/40	99 x 46	Sumico x Snk630	0,07
19/41	99 x 53	Sumico x S2K670	0,08
19/42	99 x 86	Sumico x X51Б	0,1
19/43	99 x 90	Sumico x Chk. giant	0,25
19/44	41 x 38	PR63LLO1 x Rezon	0,47
19/45	33 x 41	62/3 x PR63LLO1	1
19/46	33 x 52	62/3 x JG3	0,78
19/47	52 x 33	JG3 x 62/3	0,56
19/48	52 x 41	JG3 x PR63LLO1	1,1
19/49	72 x 33	Zorya x 62/3	0,81

19/50	72 x 41	Zorya x PR63LLO1	0,44
19/51	85 x 41	715 (USA) x PR63LLO1	0,67
19/52	85 x 61	715 (USA) x Slobozhankyi	0,53
19/53	85 x 99	715 (USA) x Sumico	0,34
19/54	104 x 52	STH-16004 x JG3	1,22
19/55	104 x 82	STH-16004 x 712 (USA)	0,59
19/57	31 x 37	56/3 x Polar CL	0,09
19/63	61 x 99	Slobozhankyi x Sumico	0,08
19/64	17 x 99	64/3 x Sumico	0,14
19/66	51 x 46	JB231AC x Snk630	0,19
19/67	51 x 86	JB231AC x X51Б	0,05
19/69	72 x 85	Зоря x 715 (USA)	0,67
19/70	33 x 72	62/3 x Zorya	0,67
19/71	41 x 33	PR63LLO1 x 62/3	0,57
19/72	41 x 72	PR63LLO1 x Zorya	0,19
19/73	61 x 37	Slobozhankyi x Polar CL	0,34
19/74	61 x L25	Slobozhankyi x L25	0,15
19/75	93 x L25	UE*1021 x L25	0,59
19/76	73 x 41	Stadion x PR63LLO1	0,53
19/77	86 x 17	X51B x 64/3	0,28
19/78	86 x 31	X51B x 56/3	0,28
19/79	86 x 53	X51B x S2K670	0,33
19/80	86 x 90	X51B x Chk. giant	0,36

The minimum content of cadmium in the seeds (less than or equal to 0,1 mg/g) was noted in 14 hybrids or 19,2% of the total amount. The cadmium content in 16 hybrids (or 21,9%) did not exceed the natural background of this element in the soil (0,21 mg/kg). The largest part of the obtained hybrids, namely 58,9%, had the characteristics of accumulating plants.

Within the last group of 5 hybrids – 19/15, 19/48, 19/54, 19/26, and 19/11, the cadmium content was 1,0 mg/kg higher. That is, it exceeded the natural background by 4,5 times. The largest share of hybrids with the minimal cadmium content (higher or equal to 0,1) was obtained in combinations with line 56\3 and commercial hybrids Rezon and Sumico as the maternal component. The minimum content of cadmium was noted in combinations with the parent component (pollinator) of commercial hybrid Sumico with X51B and JB231AC lines.

3.5.1 Characteristics of hybrids according to the breeding-controlled traits

Currently, sunflower is considered mainly as technical oil crop, which involves the processing at specialized enterprises. This, in turn, involves the possibility of controlling oil quality indicators, both due to the concentration of heavy metals in the process of buying the crop and the mixing of different batches of seeds during its processing. Moreover, significant amounts of sunflower oil are used as a raw material to produce natural lubricants, varnishes, and paints where the control of the cadmium content is less strict.

Under these conditions, the indicator of cadmium content in the original breeding material cannot be considered separately from the complex of other selection-valuable traits. Table 3,4 and Appendix C present data on the average values and limits of variation of the main indicators of the created hybrids.

An important indicator of the adaptability of sunflower hybrids to specific soil and climatic conditions is the correspondence of the genotype to the length of the growing season and the plant morphotype according to technological parameters. In the conditions under consideration, the range of trait values of the duration of the “seedling-flowering” interphase period varies from 49 to 66 days. The insignificant range of variability of the plant height trait from 123,3 to 208,2 cm indicates the absence of genotypes with characteristics of dwarfism or gigantism.

Table 3.4

Average values and variation limits of the main selection-controlled traits of inter-varietal sunflower hybrids, (2020-2021).

Trait	Limits		X	Asymmetry	Kv, %
	min	max			
Cd content in the seeds, mg/kg	0,05	2,24	0,39	2,77	98,12
Length of seedling-flowering phase, days	49,00	66,00	57,82	0,07	6,3
Height, cm	123,30	208,20	159,40	0,41	12,29
Head diameter, cm	8,60	22,70	14,40	0,64	17,83
Leaf number, pcs	11,70	36,20	18,98	1,14	27,89
Leaf of one area, см^2	120,00	387,00	251,21	0,22	24,5
Leaf area, m^2/plant	0,19	1,01	0,48	0,79	38,62
LAR, dm^2/g	0,46	5,53	1,60	2,05	60,5
1000 seed weight, g	29,70	63,40	45,46	-0,11	20,02
Seed number, pcs	257,99	1561,10	780,25	0,25	35,82
Productivity, g/plant	10,50	62,60	34,95	0,40	38,07

The average value of this indicator, (159,4 cm), is close to the hybrid parameters oriented to the zone of the central and northern Forest Steppe of Ukraine. Average indicator of the head diameter (14,4 cm), the leaf number (18,9 pcs.), and the leaf area surface per plant (0,48 m^2) are close to the morphotype of the most common sunflower genotypes in these zones.

The main components of plant productivity are the number of seeds and the weight of 1000 seeds. According to the results of the 2-year testing, the average value of the 1000 seed weight was 45,46 g with a range of variability from 29,7 to 63,4. A negative value of the asymmetry index - 0,11 indicates a leftward shift of the frequency distribution, i. e., a predominance of genotypes with a trait close to the minimum values. In terms of specific hybrids, the minimum value was noted in the 19/19 hybrid (combination 38x86). The maximum value in the hybrid is 19/78 (combination 86x31) where the trait was inherited by the type of heterosis.

According to the indicator of the seed number of, the minimum value is 257,9 pcs. was noted in hybrid of 19/31 obtained in combination 53x99. The maximum value of this indicator (1561,1) was recorded in the 19/31 hybrid, the 53x99 combination. The value of the average indicator was 780,25 with some rightward frequency shift.

The average value of the variation coefficient of 35,8% indicates that the number of seeds in the head is the main factor in the variability of productivity traits. The range of values of the productivity trait was 10,5-69,3 g. The minimum value was in the hybrid of 19/31 obtained in the combination of 53x99 with the inheritance of the trait according to the type of depression. The maximum value was in the 19/71 hybrid obtained in the 41x33 combination. It is worth noting that the productivity indicator for the best of the parents in this crossing was only 36,8 g.

Sunflower crop is characterized by the ability to regulate the indicators of the leaf surface area due to the death of the lower tiers of leaves, which are not very effective in creating the products of photosynthesis. However, in some cases, an insufficient level of drought resistance of genotypes, a low proportion of shade-tolerant chlorophyll “b” or reduced resistance to damage by rust (*Puccinia helianthi*) can trigger similar processes of reducing the plant leaf surface.

LAR is a complex parameter that characterizes the level of seed support with leaf cover. According to the test results, the value of the variation coefficient of this indicator was 60,5% with a range of variability from 0,46 to 5,53 dm^2/g. The level of correlation between the main features of the created collection of hybrids is presented in Table 3.5.

The highest level of correlation and the largest number of statistically significant relationships was noted for indicators of leaf surface area, plant productivity, and LAR, which generally correspond to the correlation structure of the original collection. However, in contrast to the original collection, the presence of statistically significant correlations was noted for the indicator of cadmium content in the seeds. This is the correlation between the index of cadmium content and the 1000 seed weight (r = 0,33), and the trait of plant seed productivity (r = 0,27). The existence of positive correlations, but of average strength, between the index of cadmium content in the seeds and the main generative parameters of plants indicates the need to differentiate the obtained collection into specialized groups.

Table 3.5

Correlation matrix of the main selection traits of the collection of inter-varietal hybrids (2020 -2021)

Trait	Cd content in the seeds, mg/kg	Length of seedling-flowering phase, days	Height, cm	Head diameter, cm	Leaf number, pcs	Leaf of one area, см^2	Leaf of one area, см^2	LAR, dm^2/g	1000 seed weight	Seed number, g
Cd content in seeds, mg/kg										
Length of seedling-flowering phase, days	0,14									
Height, cm	0,03	0,43*								
Head diameter, cm	0,02	-0,17	0,13							
Leaf number, pcs	0,04	0,35*	0,57*	0,05						
Leaf of one area, см^2	-0,13	0,05	0,28*	0,19	0,10					
Leaf area, m^2/plant	-0,03	0,27*	0,59*	0,19	0,71*	0,74*				
LAR, dm^2/g	-0,17	0,19	0,40*	-0,24	0,36*	0,50*	0,62*			
1000 seed weight	0,33*	0,34*	0,53*	0,28*	0,26	0,14	0,36*	0,04		
Seed number, g	0,08	-0,09	-0,19	0,45*	-0,03	-0,02	-0,09	-0,65*	-0,21	
Productivity, g/plant	0,27*	0,08	0,10	0,61*	0,08	0,06	0,09	-0,61*	0,38*	0,81*

**- statistically significant*

3.5.2 Correlation and the scheme of crop formation

The availability of source material with a sufficient range of variability for the main selection-controlled traits implies the development of appropriate selection programs for improving and optimizing the parameters of the new genotype. The development of a selection program requires studying the structure of the new collection, distinguishing groups according to the main directions of selection, and determining group correlations and algorithms for the formation of plant productivity. The study of the correlation scheme in hybrids with a minimum cadmium content (less or equal to 0,1 mg/kg) indicated the presence of several group differences (Table. 3.6.).

The selected group was characterized by a specific scheme of correlations between traits. The cadmium content indicator in seeds had a statistically significant correlation with LAR (r = 0,55) and had a negative correlation with the trait of plant productivity (r = - 0,66). The latter indicator is characterized by the highest level of correlation between the parameters, in particular, a positive close relationship with the head diameter (r = 0,89) and the number of seeds (r = 0,79). An important characteristic of the group was a high, negative correlation between plant productivity and LAR (r = - 0,81).

Table 3.6

Correlation matrix of the main selection traits of inter-varietal hybrids with minimal cadmium content

Trait	Cd content in the seeds, mg/kg	Length of seedling-flowering phase, days	Height, cm	Head diameter, cm	Leaf number, pcs	Leaf of one area, см^2	Leaf of one area, см^2	LAR, dm^2/g	1000 seed weight	Seed number, g
Cd content in seeds, mg/kg										
Length of seedling-flowering phase, days	0,14									
Height, cm	-0,34	0,11								
Head diameter, cm	-0,66	-0,15	0,44							
Leaf number, pcs	0,50	0,34	0,34	-0,40						
Leaf of one area см^2	0,08	-0,11	0,06	0,02	-0,03					
Leaf area, m^2/plant	0,37	0,08	0,28	-0,25	0,58*	0,79*				
LAR, dm^2/g	0,55*	-0,19	-0,14	-0,71	0,44	0,16	0,40			
1000 seed weight	-0,46	0,40	0,79*	0,26	0,30	0,03	0,18	-0,06		
Seed number, g	-0,43	-0,16	-0,02	0,77	-0,65*	0,07	-0,34	-0,86*	-0,23	
Productivity, g/plant	-0,66*	0,09	0,50*	0,89*	-0,42	0,10	-0,19	-0,81*	0,41	0,79*

**- statistically significant*

An integral part of the future selection program is to determine the algorithm for the formation of plant productivity based on the multiple regression method, Table. 3.7.

Table 3.7

Multiple regression model of plant yield capacity of inter-varietal sunflower hybrids, 2020-2021.

Regression equation	Coefficient of determination	p – level
$Z = 48,30 – 0,12*L + 0,65*X + 1,04*Y$	$R^2 = 0,98$	L = 0,09; X = 0,00; Y = 0,00.
Z – plant productivity, г; **L** – LAR, dm^2/g; **X** – 1000 seed weight, g; Y – seed number, pcs/plant.		

The developed model and the dispersion analysis showed the dominant role of the seed number in the formation of plant productivity. The index of 1000 seed weight had a smaller effect on the change in plant productivity values within the selected group of hybrids. The influence of the factor of seed supporting with leaf surface, – LAR – was the smallest, with a low level of statistical reliability. The relationship between the 1000 seed weight, the seed number and productivity are presented in Fig. 3.11.

The analysis shows that the condition of average and higher plant productivity (> 30 g) is the ability to form an inflorescence with 800 or more seeds. Only under these conditions, an increase in the 1000 seed weight significantly impacts productivity dynamics. The zone of the maximum estimated productivity (> 60 g) is determined by the presence of at least 1200 seeds in the inflorescence with 1000 seed weights of more than 50 g. Despite the lack of a recreational selection direction in modern sunflower crop, the study of crop formation features in the group of hybrids with an increased ability to accumulate cadmium contributes to the formation of a scientific basis for selecting with controlled indicators of the content of heavy metals. In contrast to the previous group of hybrids, the group with increased cadmium content in the seeds implemented a “vegetative strategy”.

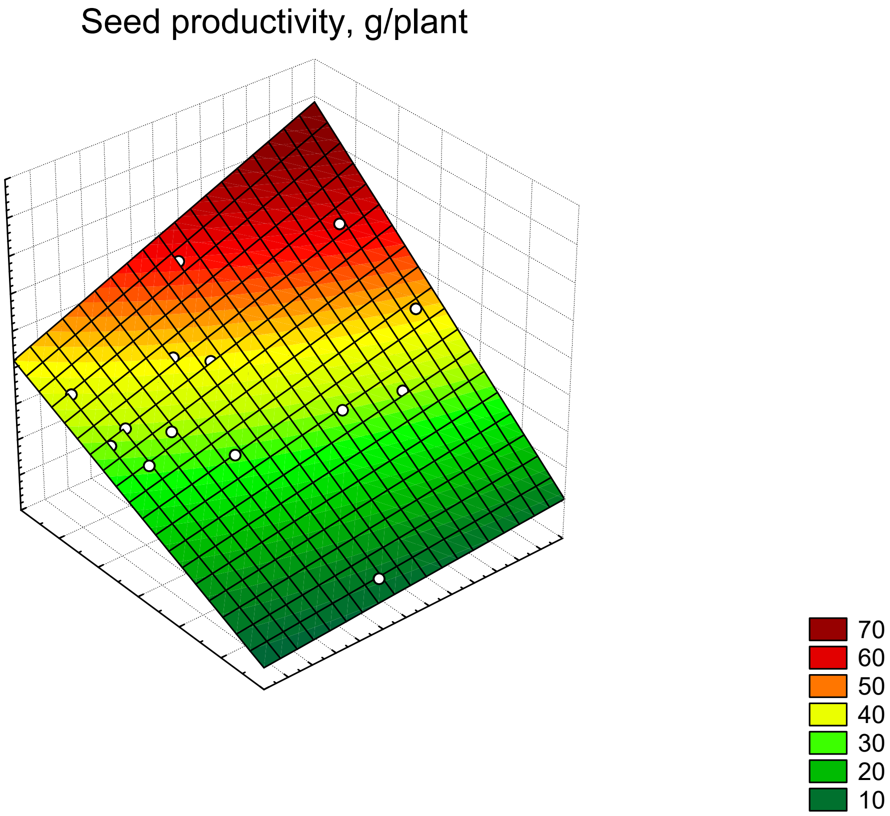

Fig. 3.11. The graph of the dependence of plant productivity on the weight of 1000 seeds and the seed number for the group of hybrids with minimal cadmium content , 2020-2021.

The parameters of LAR, number, and area of leaves had the highest level of correlation. However, the plant productivity indicator had high and statistically significant correlations with the inflorescence diameter (r = 0,75) and the number of seeds (r = 0,73), (Table. 3.8.)

Table 3.8

Correlation matrix of the main selection traits of inter-varietal hybrids with minimal cadmium content (2020-2021)

Trait	Cd content in the seeds, mg/kg	Length of seedling-flowering phase, days	Height, cm	Head diameter, cm	Leaf number, pcs	Leaf of one area, см^2	Leaf of one area, см^2	LAR, dm^2/g	1000 seed weight	Seed number, g
Cd content in seeds, mg/kg										
Length of seedling-flowering phase, days	-0,14									
Height, cm	-0,44	0,38								
Head diameter, cm	0,02	-0,41	-0,14							
Leaf number, pcs	-0,54*	0,41	0,51*	-0,31						
Leaf of one area, см^2	-0,02	-0,08	0,01	0,28	-0,08					
Leaf area, m^2/plant	-0,40	0,23	0,40	0,04	0,61*	0,72*				
LAR, dm^2/g	-0,39	0,36	0,57*	-0,35	0,60*	0,52*	0,85*			
1000 seed weight	0,17	0,14	0,15	0,53*	-0,40	0,44	0,12	-0,02		
Seed number, g	-0,05	-0,37	-0,29	0,32	0,12	-0,07	-0,04	-0,39	-0,45	
Productivity, g/plant	0,10	-0,34	-0,25	0,75*	-0,24	0,25	0,01	-0,47	0,27	0,73*

Regarding the indicator of cadmium content in the seeds, only one statistically significant correlation was noted in this group, namely with the indicator of the leaves number (r = 0,54). The inverse correlation with the plant height indicator (r = - 0,44) was also close to a statistically significant level.

More clearly, the algorithm of forming plant productivity in the group of hybrids with the maximum content is demonstrated by the results of multiple regression (Table 3.9).

Table 3.9

A multiple regression model of plant productivity of inter-varietal sunflower hybrids, 2020-2021.

Regression equation	Coefficient of determination	p – level
$Z = - 48{,}53 – 0{,}06*L + 0{,}72*X + 1{,}03*Y$	$R^2 =0{,}98$	L = 0,08. X = 0,00. Y = 0,00.
Z – plant productivity, г; **L** – LAR, dm^2/g; **X** – 1000 seed weight, g; Y – seed number, pcs/plant.		

With a general similarity to the regression model of the previous group, a higher and negative value of the free term indicates greater stability of the model. The latter is enhanced due to the indicator of the supply of seeds with a leaf surface (LAR). The high value of the determination coefficient of the calculated model is based on significant indicators of the reliability of the weight and seed number (Fig. 3.12)

The analysis of the graph indicates a significantly wider range of indicators of the number and weight of 1000 seeds. The location of the response area indicates that the average and close to an average level of plant productivity can be maintained due to high values of the 1000 seed weight at minimum relative values of the seed number.

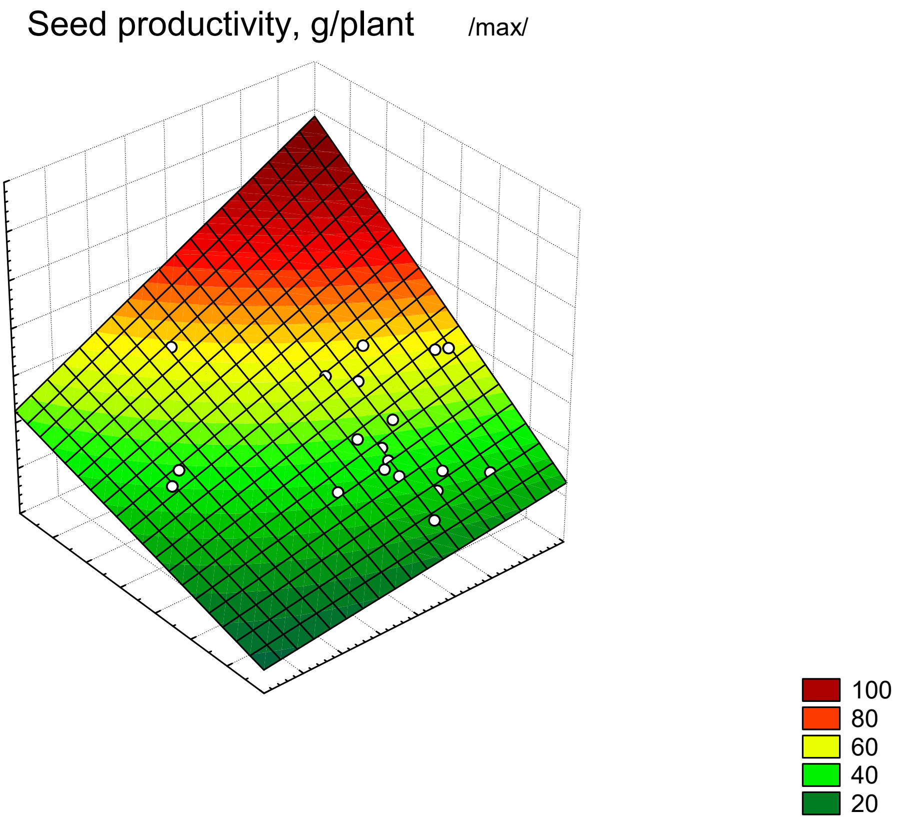

Fig. 3.12. The graph of the dependence of the plant productivity indicator on the 1000 seed weight and the seed number for the hybrid group with the maximum cadmium content, 2020-2021.

Concurrently, the maximum level of productivity (> 60 g/plant) can be ensured both due to high values of the seed number and the weight indicator of the 1000 seed weight.

Conclusions to Chapter 3.

In general, in the first stage of the research (in the vegetation experiment codition), a basic collection was formed, and 104 selected samples were analyzed. Most of the samples were provided by the National Central Research and Development Center of Ukraine, Sumy National Agrarian University, and the Institute of Oil Crops of the National Academy of Sciences.

By geographic origin, 90% of the samples were represented by the selection centers of Ukraine and the USA. Individual samples represented Bulgaria, Cuba, Spain, Kazakhstan, and Moldova.

According to the results of a vegetation experiment using an analytical background with a cadmium concentration of 1,0 mg/kg, it was established that the range of values of the concentration indicator of this metal in the aboveground part of plants varied from 0,5 to 2,6 mg/kg. High resistance to cadmium accumulation in the aboveground part of plants (0,5 to 0,9 mg/kg) was noted in 11 samples: JB231AC, S2K670, UE0100938, IPS\125, 64\3, Polaris CL, Reason, 56\3, Snk630, UE0100977, and UE0100018. The share of this group in the total collection was 10,6%. The group with a very low level of resistance (cadmium content is more than 2,11 mg/kg) included 7 selection samples such as PR63LL01, UE0100056, UE0100715, 62\3, UE0100052, JG3, and UE0100060. The share of this group in the collection was 6,7%. According to the results of a 3-year experiment (natural cadmium background is 0,21 mg/kg), indicators of cadmium content in the seeds of 95 selection samples of the basic collection were determined.

The average value was 0,34±0,01 with a range of variability of the indicator of 0,23 – 0,43 mg/kg. The minimum level of cadmium accumulation in the seeds was noted in 16 samples, including 64\3, JB231AS, 56\3, Neon-2011, UE0100938, UE0100018, UE0100977, S2K670, 62\3, Snk630 Sumico, Polaris CL, and Rezon.

A comparison of the data from the vegetation experiment and the field experiment regarding the indicators of cadmium content in the seeds showed the similarity of the results regarding the differentiation of the collection according to the level of resistance to cadmium accumulation. On average, for the collection, 63,8% of the selection samples analyzed by the method of indirect evaluation in the vegetation experiment corresponded to the indicators obtained in the field experiment. The highest rates of compliance were in the groups with unsatisfactory and low levels of resistance – 85,7 and 84,2%, respectively. A group with a high level of resistance was also characterized by a high share of common hybrids of 76,1%. Therefore, the method of indirect evaluation provides high efficiency of negative selection against the trait and can be used for the preliminary selection of samples with a high level of resistance to the accumulation of cadmium in the seeds.

According to the results of the basic collection assessment in terms of the field experiment, the average values, and the range of variation of the main selectively controlled traits were determined. The average value of the indicator of the duration of the “seedling-flowering” period was 56,5 days with a range of variability from 50 to 66 days. The coefficient of variation was 6,81%. The average value, the range of variability, and the coefficient of variation of the base collection for plant productivity were 33,6 g, 11,70 – 63,30 g, and 20,75%, respectively.

According to the results of the cluster analysis, morphological heterogeneity was established in the group of low cadmium samples, with the selection of three intragroup clusters. The first cluster contained late-ripening (the duration of the “seedling-flowering” phase was > 64 days), tall (> 193 cm), and low-yielding samples, such as UE0100114, UE01000114, UE0100977, and UE0100018.

The second cluster included samples with an average and higher than the average level of plant productivity for the collection (22,6 – 52,0 g/plant). A separate cluster was formed by the samples with specific characteristics. The heterogeneity of the group of low-cadmium samples implies the availability of different mechanisms of seed productivity formation and implementation of the trait of resistance to cadmium accumulation. On the contrary, in the structure of the samples of the “high cadmium” group, the availability of one unified mechanism of transportation of biologically active cadmium from vegetative organs to the seeds was observed.

The heterogeneity of the basic collection of selection samples and the difference in the mechanisms of ensuring a low level of cadmium accumulation in sunflower seeds was confirmed by the results of inter-varietal crossings. 73 samples of inter-varietal hybrids were obtained and tested in F_1 and F_2. The following was established: when crossing “low cadmium” parental forms, only a fifth or 19% inherited the trait of resistance to cadmium accumulation by heterosis type. The shares of hybrids that inherited the trait by the type of partial positive dominance and intermediate for parental forms amounted to 9,5% each.

In most cases, 62% of trait inheritance occurred according to the type of depression and partial negative dominance. Depression and partial negative dominance were also the predominant types of inheritance in crossing variants in the group of “high cadmium” selection samples and the intergroup crossing. The total share of these types of phenotypic inheritance was 85,7% when crossing “high cadmium” samples and 66,7% in intergroup crossings.

The average indicator of cadmium content in the hybrid seeds for the experiment was 0,41 mg/kg with a range of variation from 0,05 to 2,24 mg/kg. The minimum values were noted in samples 19/06, 19/32, and 19/67 obtained in the combinations of Rezon X PR63LLO1, Zorya X JG3, and JB231AC X X51B, respectively.

The largest share of hybrids with minimal cadmium content (bigger than 0.1) was obtained in the combinations with 56\3-line, commercial hybrids Rezon, and Sumico as the maternal component. The commercial hybrid Sumico, lines X51B, and JB231AC were used as the parent component (pollinator).

Based on the results of crossing, the range of variability of other selectively controlled traits, namely the duration of the “seedling-flowering” period, the weight of 1000 seeds, and the plant productivity index, was expanded. The range of values of the productivity indicator was 10,5-69,3 g. The maximum value was the hybrid 19/71 obtained in the combination 41x33.

Differentiation of the collection of hybrids based on the cadmium content in the seeds ensured the formation of groups with different schemes of correlations of this characteristic. In the group of low cadmium hybrids, the indicator of cadmium content in the seeds was statistically significantly correlated with LAR (r = 0,55) and had a negative correlation with the indicator of plant productivity (r = - 0,66).

The last indicator was characterized by the highest level of correlation between other selectively controlled ones, such as the head diameter (r = 0,89) and the seed number (r = 0,79). An important characteristic of the group was the high, negative correlation between plant productivity indicators and the LAR indicator (r = - 0,81). In the groups of hybrids, the group with increased content of cadmium in the seeds implemented a “vegetative strategy” with a high level of distortion of the LAR parameter and indicators of the number and area of leaves.

CHAPTER 4

STUDY OF MANIFESTATION MECHANISMS AND CONTROL OF SIGNS RESISTANCE TO CADMIUM ACCUMULATION

4.1 Physiological comparison of the two sunflower varieties

Cadmium (Cd) is a non-essential heavy metal element for plants and animals, which not only inhibits the growth and development of plants but also seriously threatens human health through the food chains (Li et al., 2019c; Maria Celeste et al., 2013). In recent years, with the rapid development of mining, industrial, and agricultural production, heavy metal soil pollution has become increasingly serious because of human activities, such as the release of industrial wastewater, waste gas discharge, sewage irrigation, and the abuse of chemical fertilizers and pesticides (Yan et al., 2021b). Cd has the characteristics of being easily absorbed and accumulated in plants (Chen et al., 2018; Shahid et al., 2017), and soil Cd pollution seriously affects crop quality and safety production. To reduce soil Cd pollution in crops, selectors carried out the genetic improvement screening of soil metal hyperaccumulators and low Cd accumulation crops.

Sedum alfredii (Hu et al., 2019b), *Solanum nigrum* (Dou et al., 2020), and *Bidens pilosa* (Dou et al., 2019) have been studied extensively as hyperaccumulators based on their ability to grow in Cd-rich soils and to accumulate large amounts of Cd in plants. Besides, cultivating low Cd crop varieties is one of the most effective ways of reducing this human health risk, and a great deal of research has been carried out on the crops, such as rice (Liu et al., 2020a; Tefera et al., 2021; Wang et al., 2021b) and wheat (Liu et al., 2020d; Zhang et al., 2020e). However, understanding of the mechanism of Cd detoxification and accumulation in plants is currently limited, which hinders further development and application of screening of hyperaccumulators and low Cd accumulation varieties.

Cd poses significant problems for successful seed germination and establishment. Under heavy metal stress, plants generally appear short stature, root growth retarded, and leaves curled and withered. Fresh and dry masses of crop seedlings decreased significantly under 80 μM Cd stress (Chen et al., 2017). Cotyledons and the emergence of young leaves of mung bean were reduced by 13% and 74%, respectively, under Cd stress, compared with control plants, root length and root surface area were significantly reduced, and plant height and stem diameter consistently decreased with an increase in Cd concentration (Rashid et al., 2021). Lv et al. (2019) showed that Cd stress reduced the biomass of aboveground and root tissue of rice. The stem fresh weight of the rice variety of NJ6 in the 10 μM Cd and 50 μM Cd groups decreased by 59,6% and 42,3%, respectively, and that of 'Y32' decreased by 39,5% and 36,8%, respectively, relative to no-Cd control plants.

At concentrations, Cd toxicity can seriously affect plant metabolism, respiration, photosynthesis, transportation, and growth, reducing root activity, slowing seedling growth, and eventually leading to plant death (Ahmad et al., 2015; Zhang et al., 2014).

High Cd concentration mainly destroys chloroplast structure and inhibits chlorophyll synthesis in plant leaves, causes changes in the ratio of chlorophyll A and B, inhibits PS and PS , and catches light pigment-protein complex formation or makes its disintegration, affects the ability of the photosynthetic electron transport, and lowers the net photosynthetic rate, thus inhibits the photosynthesis in plants.

High Cd concentration can inhibit the activities of related enzymes in plant respiration, which will block the starch degradation process and the electron transport in the respiratory chain, leading to the decrease of respiration efficiency of various organs. Cd stress also reduced the activities of amylase, dehydrogenase, and nitrate reductase in young plants, which seriously interfered with the normal metabolism of plant cells.

Plants respond to Cd stress though adjusting their own physiological and biochemical processes, among which the accumulation and subsequent detoxification of reactive oxygen species (ROS) caused by heavy metals is an important defense response (Zhang et al., 2020a). To reduce the oxidative damage caused by the excessive ROS induced by Cd stress, plants have evolved antioxidant enzyme and non-enzyme systems during the long-term phylogenetic process.

Antioxidant enzymes mainly include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR), etc. Non-enzymatic systems mainly consist of reduced glutathione (GSH) and ascorbic acid (AsA), etc. (Wang et al., 2018). Under the regulation of the antioxidant enzyme defense system, plants can maintain normal growth and development in a certain range of heavy metal concentrations.

Sunflower (*Helianthus annuus* L.) is a food and bioenergy product and belongs to *Asteraceae* family (Bashir et al., 2021). It is used as a phytoremediator due to its large biomass, fast growth, and high tolerance to heavy metals (Bayat et al., 2021b; Benavides et al., 2021; Tang et al., 2003). Nowadays, few Physiological studies have been done on sunflowers.

In our study, two sunflower varieties of 62\3 and JB231AC were chosen as high/low Cd accumulators, comparing physiological analyses between them under different Cd stress. The study provides important information for further research on Cd accumulation and detoxification mechanism in sunflowers. (Fu et al., 2022)

4.1.1 Comparison of phenotype characteristics to Cd stress between high/low Cd sunflower varieties

To examine the plant growth difference between 62\3 and JB231AC under Cd stress, the plant growth parameters were investigated. With the increase in Cd concentration, the seedling of both varieties showed gradually weaker growth potential and became yellow (Fig. 4.1), and 62\3 almost wilted and died under 100μM Cd stress. The result showed that 62\3 was sensitive to Cd toxicity, that is, JB231AC had better Cd tolerance than 62\3.

The Cd concentration in the roots was much higher than that in the shoots in both varieties, and the Cd concentration in the roots and shoots of 62\3 higher than that in JB231AC, especially under 100μM Cd stress. The Cd TF value of 62\3 was higher than that of JB231AC in the range of Cd concentration from 0 to 50μM, and there was no significant difference between the two varieties under the same concentration of Cd stress

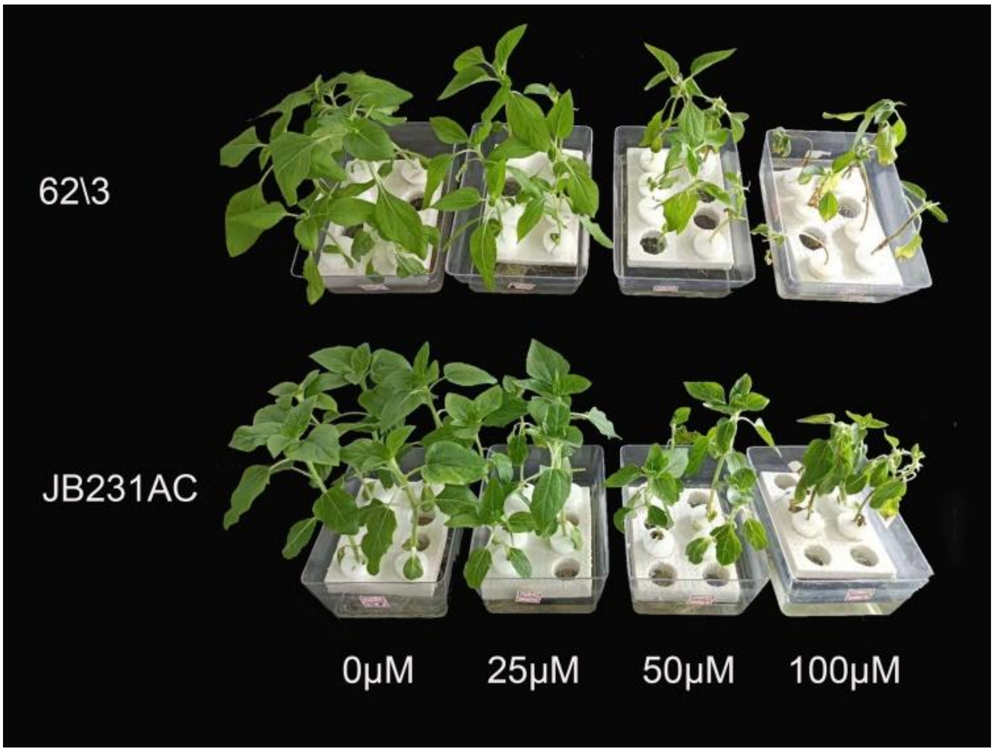

Fig. 4.1. Performance of growth and Cd accumulation of the 62\3 and JB231AC genotypes.

The result showed that 62\3 was a high Cd accumulation variety and had a higher root-to-shoot Cd translocation ratio than JB231AC (Fig. 4.2).

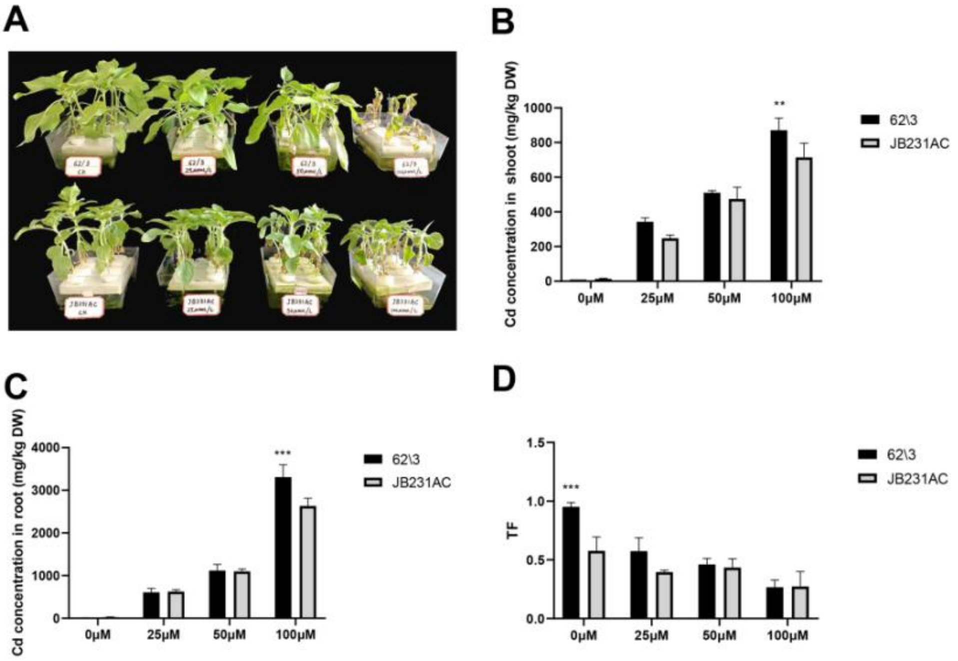

Fig. 4.2. Performance of growth and Cd accumulation of 623 and JB231AC genotypes.

The plant's high and fresh weight of 62\3 and JB231AC decreased with the increase of Cd concentration, but there was no difference between the two varieties at the same concentration of Cd stress. The dry weight of the shoot and root of 62\3 and JB231AC decreased with the increase in Cd stress, but the genotype JB231AC had bigger biomass than 62\3 at the same concentration of Cd stress. (Fig. 4.3)

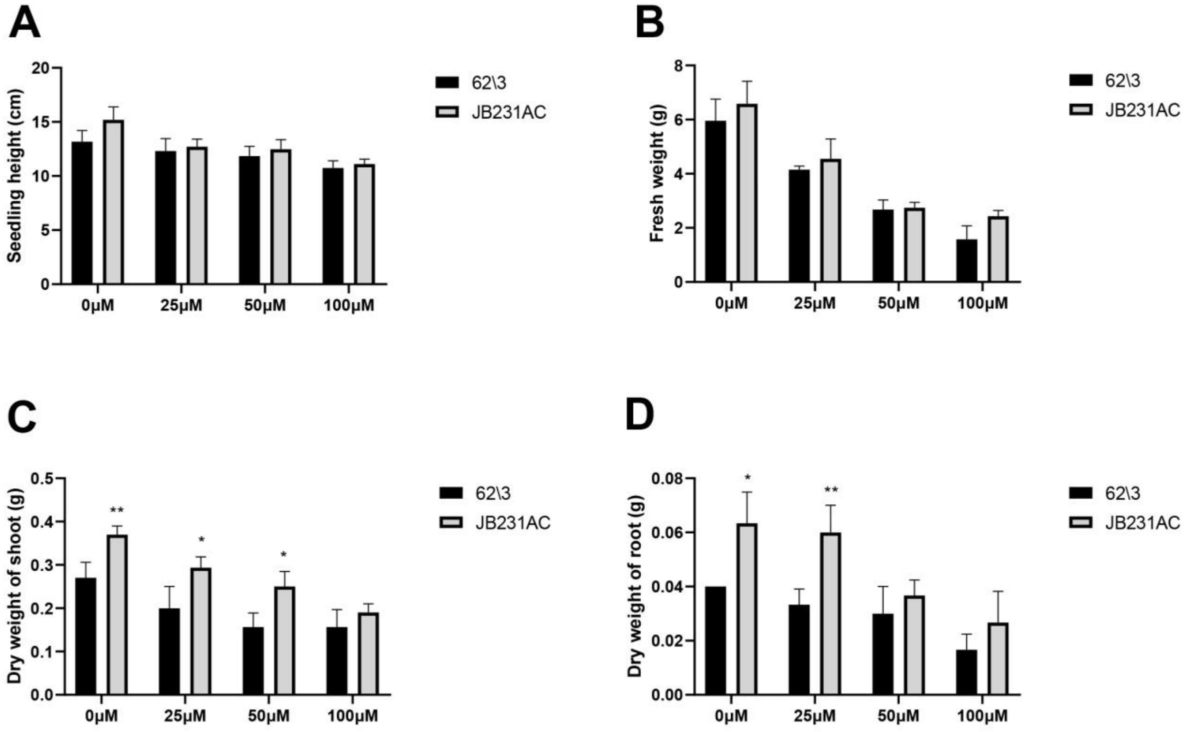

Fig. 4.3. Vegetative performance of 623 and JB231AC.

The statistical analyses were conducted using all the performance data of the 62\3 and JB231AC under Cd stress. Data presented are the means (n=3), and error bars denote the standard deviations. The asterisk represents the significant difference between 62\3 and JB231AC under the same Cd stress. *: P<0,05, **: P<0,01, and ***: P<0,001.

4.1.2 Physiological responses to Cd stress in two sunflower varieties

For investigating the level of antioxidant reaction to Cd stress, the content of H_2O_2 and MDA was measured. The H_2O_2 content was higher in JB231AC than in 62\3 in the range of 0-50μM Cd stress, increased with the increase of Cd concentration, and then decreased under 100μM Cd stress. The content of MDA increased with the increase of Cd concentration and was higher in JB231AC than in 62\3 in the 0-50 μM range of Cd stress. The contents of H_2O_2 and MDA were no differences between 62\3 and JB231AC under the same concentration of Cd stress (Fig. 4.4)

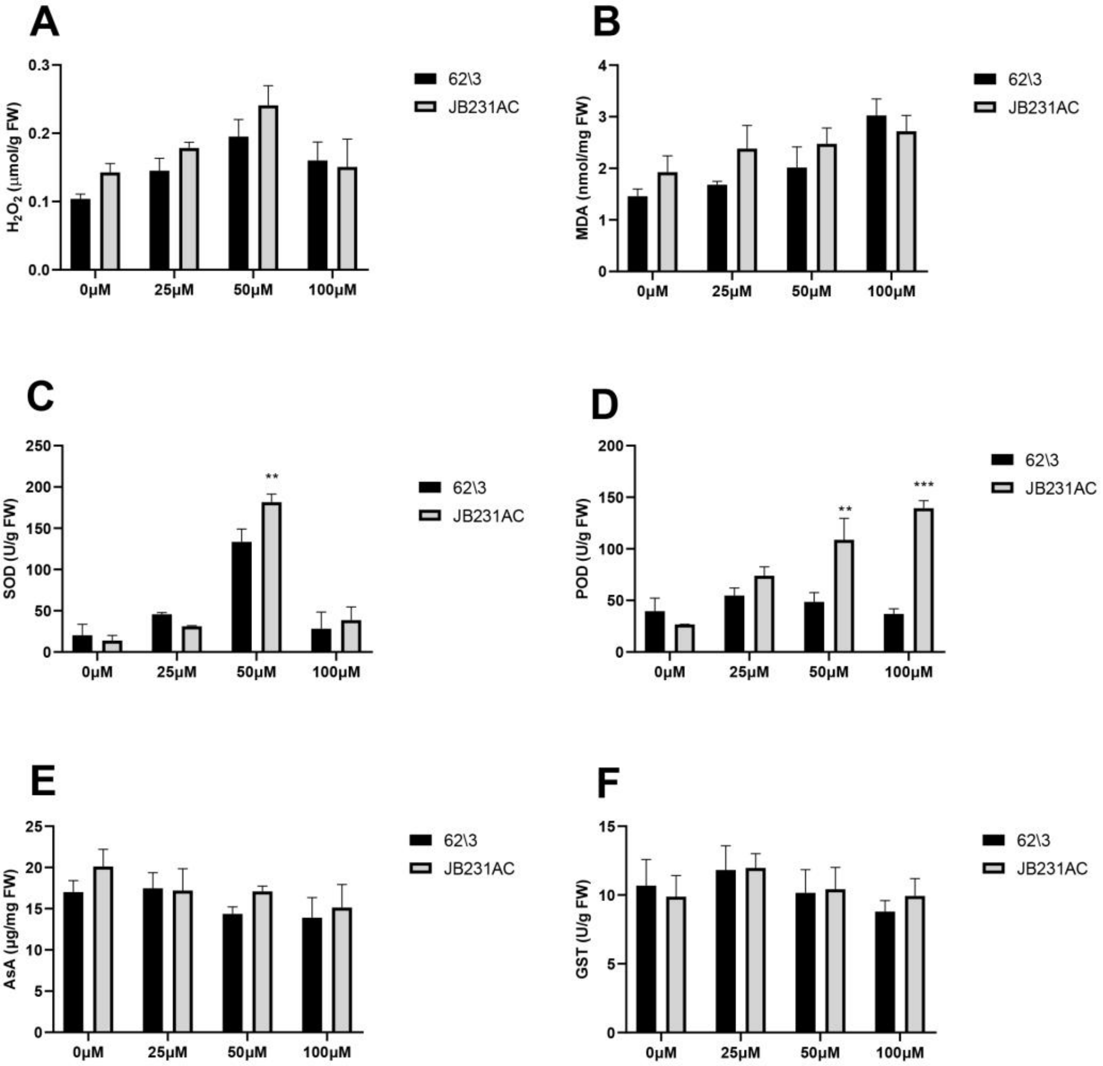

Fig. 4.4. Antioxidant systems to Cd stress between the 62\3 and JB231AC genotypes. **A** H_2O_2 content. **B** MDA: Malondialdehyde. **C** SOD: Superoxidedismutase. **D** POD: Peroxidase. **E** AsA: Ascorbic acid. **F** GST: Glutathione S-transferase. For **A-F** the statistical analyses were conducted using all the performance data of the 62\3 and JB231AC under Cd stress. Data presented are the means (n=3), and error bars denote the standard deviations. The asterisk represents the significant difference between 62\3 and JB231AC under the same Cd stress. **: P<0,01, ***: P<0,001.

The activities of SOD and POD were important indices for showing Cd stress levels in antioxidant enzyme systems. With the increase of Cd concentration, the activity of SOD first increased quickly and then decreased and reached the maximum value under 25μM Cd stress with a significant difference between 62\3 and JB231AC. Therefore, the activity of SOD was strongly stimulated under 50μM Cd stress. With the increase of Cd concentration, the activity of POD in 62\3 first increased and then decreased and reached the maximum value under 50μM Cd stress. But the activity of POD in JB231AC increased with the increase of Cd concentration. The activity of POD was higher in JB231AC than that in 62\3, showing very significant differences under 50μM and 100μM Cd stress. This result indicated that the regulation response of POD to Cd stress was different in the two varieties.

The changes in the concentration of AsA and the activities of GST were not affected in different Cd levels compared with the control, and there was no difference between varieties. The result suggested that the AsA and GST had almost no response to Cd stress in this study.

4.2 Accumulation of trace metallic elements in two sunflower varieties under Cd stress

In recent years, with the rapid development of mining, industrial, and agricultural production in the world, heavy metal soil pollution has become increasingly serious because of human activities, such as industrial wastewater, waste gas discharge, sewage irrigation, and the abuse of chemical fertilizers and pesticides (Bashir et al., 2021; Singh & Prasad, 2014; Yan et al., 2021a). Heavy metal Cd is a non-essential element for plants and animals, which inhibits the growth and development of plants (Li et al., 2019b; Maria Celeste et al., 2013). Cd in plants interferes with normal metabolism, affecting photosynthesis and respiration, reducing root activity, slowing seedling growth, making leaves small and yellow, and eventually leading to plant death (Ahmad et al., 2015; Fan et al., 2011; Jaouani et al., 2018). Consumption of Cd-contaminated plant material is one of the main sources of Cd intake for people, posing a threat to human health, even at low concentrations (Jaouani et al., 2018; Reyes-Hinojosa et al., 2019; Templeton & Liu, 2010). Cd repair and accumulation have been studied in many plant species, such as soybean (Li et al., 2012a), wheat (Chen et al., 2017c), barley (Chen et al., 2010a), rice (Prerna et al., 2020), maize (Dakak & Hassan, 2020), rapeseed (Wu et al., 2015b), and millet (Han et al., 2018b), etc.

Trace elements are widely found in nature, but their content is very low. Trace elements are found in rocks, soil, plants, and animals. Researchers have found ninety kinds of trace elements in the inorganic part of the soil. The essential trace elements in plants are copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), boron (B), and molybdenum (Mo). During the growth and development of crops, the demand for trace elements is limited, only a few parts per million to a few parts per hundred thousand of the total amounts. Therefore, soil trace elements not only have the problem of insufficient supply to plants but also have the problem of excessive supply and poisoning, thus affecting the soil environment and the growth and development of crops.

Soil trace elements are related to the parent material, soil physical and chemical properties, climate, and environment. The trace elements absorbed in crops are mainly related to the content and available state of soil trace elements, as well as related to crop types, variety characteristics, fertilization, and management measures. The available states of trace elements in black soil are strongly correlated with their contents, soil PH and Eh values, and soil organic matter content. The available states of Cu, Zn, Fe, Mn, B, and Mo in black soil are significantly correlated with the contents of trace elements. Except for Cu, available state contents of trace elements in black soil are strongly correlated with organic matter in the soil. Available state contents of B and Mo are positively correlated with soil PH, while available state contents of Cu, Zn, Fe, and Mn are negatively correlated with soil PH. The correlation analysis showed that the available state contents of Fe and Zn were significantly negatively correlated with the PH value. The regional differences in the available states of trace elements are mainly related to the parent material and soil type.

Sunflower (*Helianthus annuus* L.) is among the world's most important oilseeds. As sunflower seed oil is one of the healthiest vegetable oils available for cooking, there is increasing demand due to a health-conscious diet (Bán et al., 2021). Studies have shown that sunflower has a high enrichment capacity for heavy metals (Cornu et al., 2020; Zhou et al., 2020a). Trace metallic elements (TMEs) are indispensable elements in nature, which are lost to land through mining and production (Robert & Stengel, 1999). Compared to other pollutants, TMEs are non-biodegradable. On the one hand, certain heavy metals are necessary or even essential for living beings, though, at reasonable concentrations in certain environmental matrices. On the other hand, at a high threshold, they (Cd, Pd, and Hg) exhibit more or less strong toxicity, which strongly harms most living organisms (Aristide et al., 2021). At present, some TMEs, such as Cd (Cornu et al., 2016; Silveira et al., 2021), As (Wang et al., 2020a), Hg, Cu (Mahardika et al., 2018), and Pb (Alaboudi et al., 2018) have been studied in some crops, however, there are little studies about TMEs in sunflowers. Issam et al. (Issam et al., 2015) studied the role of selenium (Se) in regulating the oxidative stress of sunflower seed roots induced by Cd, and the results strongly suggested that exogenous selenium may improve the tolerance of plants to the oxidative stress induced by Cd. Shoot nutrient uptake of plants decreased as nickel (Ni) levels increased, and a high level of Ni decreased root colonization in sunflowers (Jarrah et al., 2019). Studies have shown that lithium (Li) and strontium (Sr) can be absorbed by the body and affect human health (Vladimir & Sofia, 2014; Zaichick et al., 2009), but little research has been done about Li or Sr in crops.

In our paper, the Cd gradient experiment was carried out on high and low Cd accumulation sunflower varieties. The growth characteristics of the two varieties under Cd stress in the seedling stage and the accumulation of TMEs Li, Ni, and Sr in the two sunflower varieties under Cd stress were studied. This paper provides a theoretical basis for the safe production of sunflowers on metal-contaminated soil. (Fu & Trotsenko, 2021a)

4.2.1 Li concentration

The Li concentration in the root and aboveground part of the two varieties was approximately the same under Cd stress, and the high Cd variety of 62\3 accumulated less Li than the low Cd variety of JB231AC (Fig. 4.5). In the root, Li accumulation was significantly or extremely significant differences when the Cd concentration was 50µM and 100µM. With the increase in Cd concentration, the Li concentration in the root increased, the change in the aboveground part was not regular, and there might be experimental error.

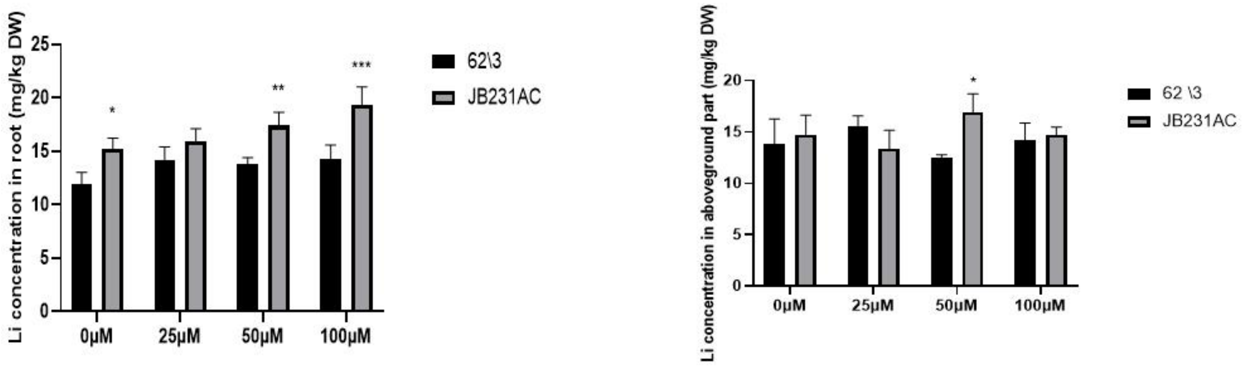

Fig. 4.5. Li accumulation in the root and the aboveground part.

4.2.2 Ni concentration

The Ni accumulation in sunflower increased firstly and then decreased with the increase in Cd concentration, and the maximum value appeared at 50µM in the root and 25µM in the aboveground part (Fig. 4.6). The Ni concentration in the high CD variety 62\3 was lower than that in the low Cd variety JB231AC, and there was a very significant difference in the Cd concentration at 25µM and 50µM in the root and aboveground parts, respectively.

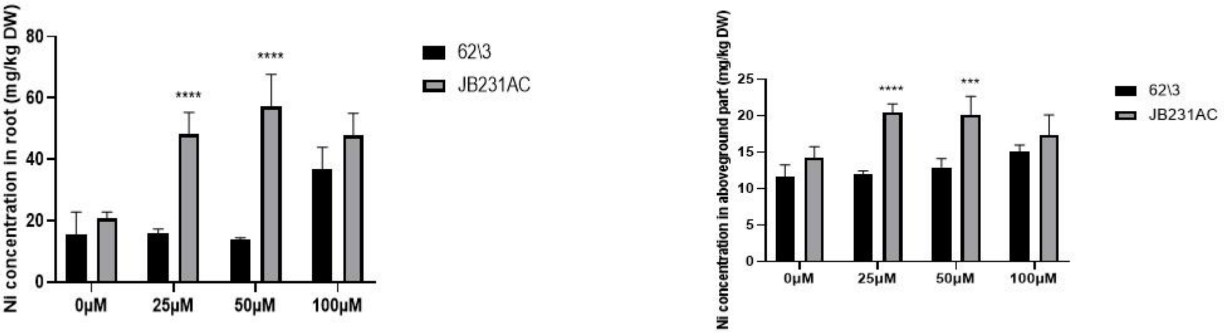

Fig. 4.6. Ni accumulation in the root and the aboveground part.

4.2.3 Sr concentration

With the increase in Cd concentration, the Sr accumulation in the root of the two sunflower varieties varied, but remained basically flat, which may be caused by experimental error, and basically showed a downward trend in the aboveground part (Fig. 4.7). Sr accumulates in a roughly equal concentration in the root and the aboveground part. In the aboveground part, the high Cd variety 62\3 accumulated relatively higher Sr concentration than the low Cd variety JB231AC.

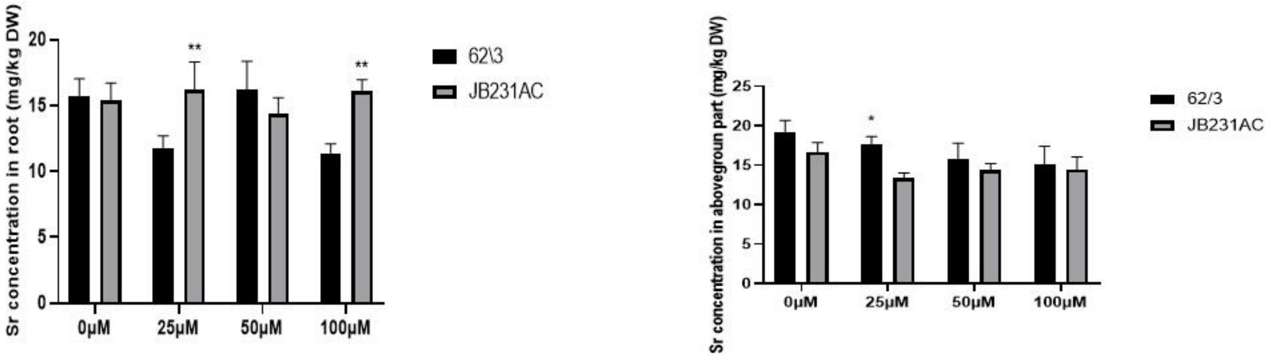

Fig. 4.7. Sr accumulation in the root and the aboveground part.

4.3 Transcriptome comparison of the two sunflower varieties with the high/low Cd accumulation

As more and more genomes have been sequenced, the understanding of crop molecular mechanisms has changed dramatically. But the huge amount of sequence information also poses new challenges to researchers. How to make use of this sequence information, how to study gene function through biochemical and molecular biological methods, to further understand the physiological processes of plants and the regulation mechanism of abiotic stress on plant growth and development are the questions to be answered.

Transcriptome sequencing technology has become the first developed and widely used technology due to its advantages of high throughput, high accuracy, high sensitivity, and no varieties limitation. Transcriptome sequencing technology provides a powerful means to study the expression patterns of single or multiple genes at specific developmental stages of an organism or in different environments.

Plants may be affected by various external adverse factors during their growth, such as heavy metals, insect pests, salt damage, drought, ozone, mechanical damage, and other biotic and abiotic stress factors, resulting in different degrees of stress on plant growth and development. Transcriptome analysis can be used to understand the expression changes of related genes in certain organisms under biotic and abiotic stress to provide a theoretical basis for elucidation of the genetic mechanism of plant response to stress.

With the advent of next-generation sequencing technologies, many transcriptomic studies on heavy metal stress have mainly focused on rice (Oono et al., 2016; Oono et al., 2014), wheat (Zhou et al., 2019a), rapeseed (Zhang et al., 2018b), *Sedum alfredii* (Yang et al., 2017), cotton (Han et al., 2019), and mustard (Zhang et al., 2021a), which revealed the molecular mechanism of transportation, accumulation, and detoxification of Cd in different plants. Two rapeseed genotypes were chosen to investigate the Cd translocation mechanism by transcriptomic comparison, and the result showed that *BnNramp2;1* and *BnNramp4;2* were two main Cd transporters (Wang et al., 2019b). Hu et al. compared the transcriptomes of difference tissue of *Sedum alfredii* under Cd stress, the result showed that ATP-binding cassette (ABC) transporters exhibited significant enrichment and accounted for approximately one-third of the total selected differentially expressed genes (Hu et al., 2019b).

Sunflower (*Helianthus annuus* L.) is a food and bioenergy product and belongs to *Asteraceae* family (Bashir et al., 2021). It is used in phytoremediation due to its large biomass, fast growth, and high tolerance to heavy metals (Bayat et al., 2021b; Benavides et al., 2021; Tang et al., 2003). Nowadays, few transcriptomic studies have been done on sunflowers.

In our study, two sunflower varieties of 62\3 and JB231AC were chosen as high/low Cd accumulators, using phenotypic, physiological, and transcriptomic analyses between them under different Cd stress. The study provides important information for further research on Cd accumulation and detoxification mechanism in sunflowers. (Fu et al., 2022)

4.3.1 RNA-seq Analyses of 62\3 and JB231AC under Cd stress

The leaves of the no-Cd control and Cd-treated (50μM $CdCl_2 \cdot 2.5H_2O$) were collected to investigate molecular responses at the transcriptional level by RNA-seq. 12 samples containing three repetitions per treatment have been processed for mRNA sequencing and 93,62Gb Clean Data with Q30 ≥ 93,90% was obtained (Table 4.1). 78,975 unigene with a total length of 59,469,664nt and an average length of 1340nt were obtained after a sequence assembly (Table 4.2). the length distribution of unigenes and a saturation test of RNA-seq data were all perfect (Fig. 4.8, Appendix F). The result of sequencing and assembly showed that the mRNA-seq Library was of high quality and could be used for transcriptome data analyses.

Table 4.1

Quality assessment of the raw data

Sample ID	ReadSum	BaseSum	GC(%)	N(%)	Q20(%)	CycleQ20(%)	Q30(%)
J-50-1	19398374	5.8E+09	45.43	0	97.9	100	94.18
J-50-2	19346886	5.8E+09	45.08	0	97.76	100	93.9
J-50-3	28973679	8.7E+09	45.27	0	98.24	100	94.82
J-CK-1	23160280	6.9E+09	45.76	0	97.91	100	94.19
J-CK-2	31483734	9.4E+09	45.68	0	97.93	100	94.13
J-CK-3	25391520	7.6E+09	46.43	0	98.15	100	94.64
S-50-1	28942916	8.7E+09	44.78	0	98.04	100	94.32
S-50-2	31785104	9.5E+09	44.79	0	97.86	100	93.96
S-50-3	28341673	8.5E+09	44.79	0	98.06	100	94.4
S-CK-1	24043045	7.2E+09	45.25	0	98.17	100	94.62
S-CK-2	22552792	6.7E+09	44.55	0	98.04	100	94.42
S-CK-3	29484716	8.8E+09	44.65	0	98.02	100	94.28
J: JB231AC; CK: control; S: 62\3. Each treatment contains 3 replications.							

Table 4.2

Statistics of assembly

Length Range	**Transcript**	**Unigene**
200-300	39,720(10.14%)	27,715(35.09%)
300-500	42,752(10.91%)	19,478(24.66%)
500-1000	78,697(20.09%)	15,085(19.10%)
1000-2000	127,651(32.58%)	9,900(12.54%)
2000+	102,966(26.28%)	6,797(8.61%)
Total Number	391,786	78,975
Total Length	579,388,054	59,469,664
N50 Length	2,089	1,340
Mean Length	1478.84	753.02

Note: The numbers in the table are the number of unigenes that fall into the length range. The percentage in the brackets stands for the proportion of unigenes with a certain length in total unigenes. The total number is the number of all unigenes obtained from the assembly. N50 length is the median length of all unigenes. Mean length is the average length of all unigenes

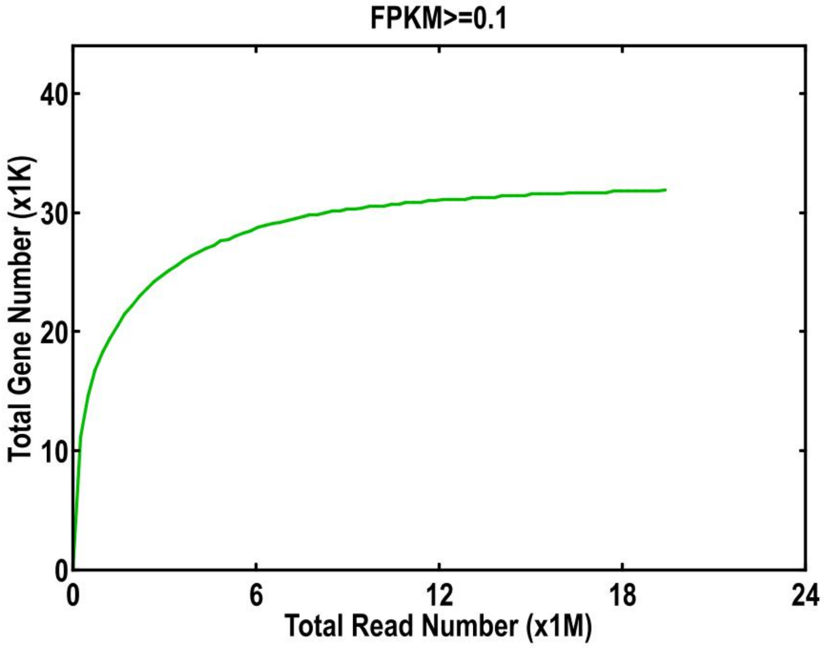

Fig. 4.8. Saturation test of RNA-Seq data.

4.3.2 Identification of Cd-regulated differential expression of genes (DEGs)

For further elucidating molecular mechanism, the high-throughput DEGs sequencing between 62\3 and JB231AC under Cd stress was performed by DESeq R package (1.10.1). the fold change was (FC) ≥2 and the criteria of false discovery rate (FDR)<0,01 were the threshold values. A total of 8818 DEGs were identified, among them, 3196, 4769, and 4764 DEGs were identified in the three comparison groups of 62\3 (CK vs. 50μM), JB231AC (CK vs. 50μM), and 62\3 vs. JB231AC (50μM Cd), respectively. There were 650 co-expressed DEGs in the three groups. More DEGs were identified in JB231AC (CK vs. 50μM) than those in 62\3 (CK vs. 50μM), and the two varieties had more up-regulated DEGs than the down-regulated. Comparing 62\3 with JB231AC under 50μM Cd stress, there were approximately equal numbers of up- and down-regulated DEGs. (Fig. 4.9-10)

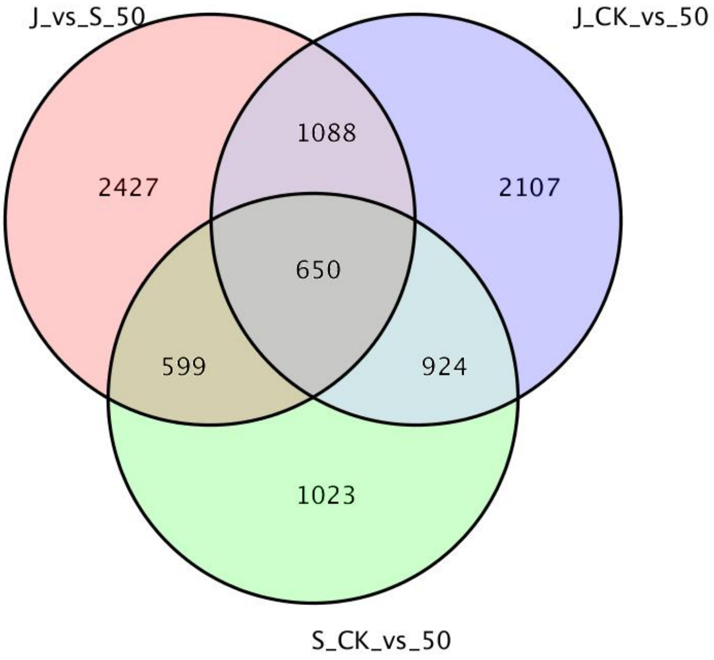

Fig. 4.9. Venn diagrams showing all DEGs shared among the three groups.
J: 62\3, S: JB231AC, CK: control, 50: 50μM CdCl2•2,5H2O stress, vs: versus.

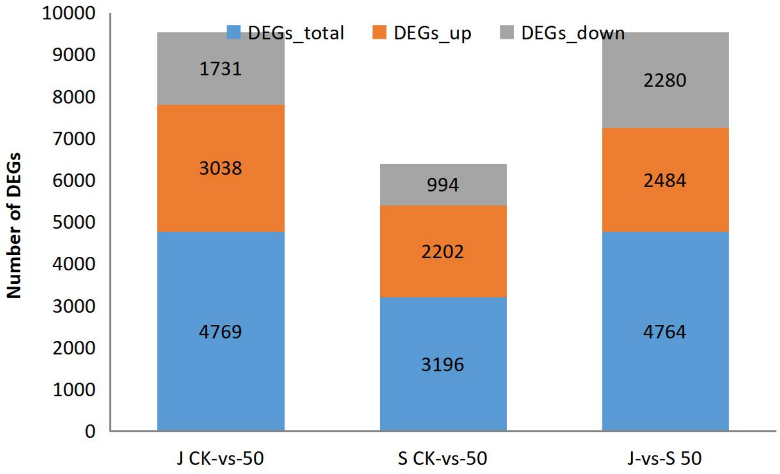

Fig. 4.10. Number of DEGs that were up- or down-regulated in the three groups. J: 62\3, S: JB231AC, CK: control, 50: 50μM $CdCl_2$•2,5H_2O stress, vs: versus.

4.3.3 Functional annotations of Cd- regulated DEGs

GO class analysis was used to identify the function annotation of 4764 DEGs between 62\3 and JB231AC under 50μM Cd stress (Fig. 4.11).

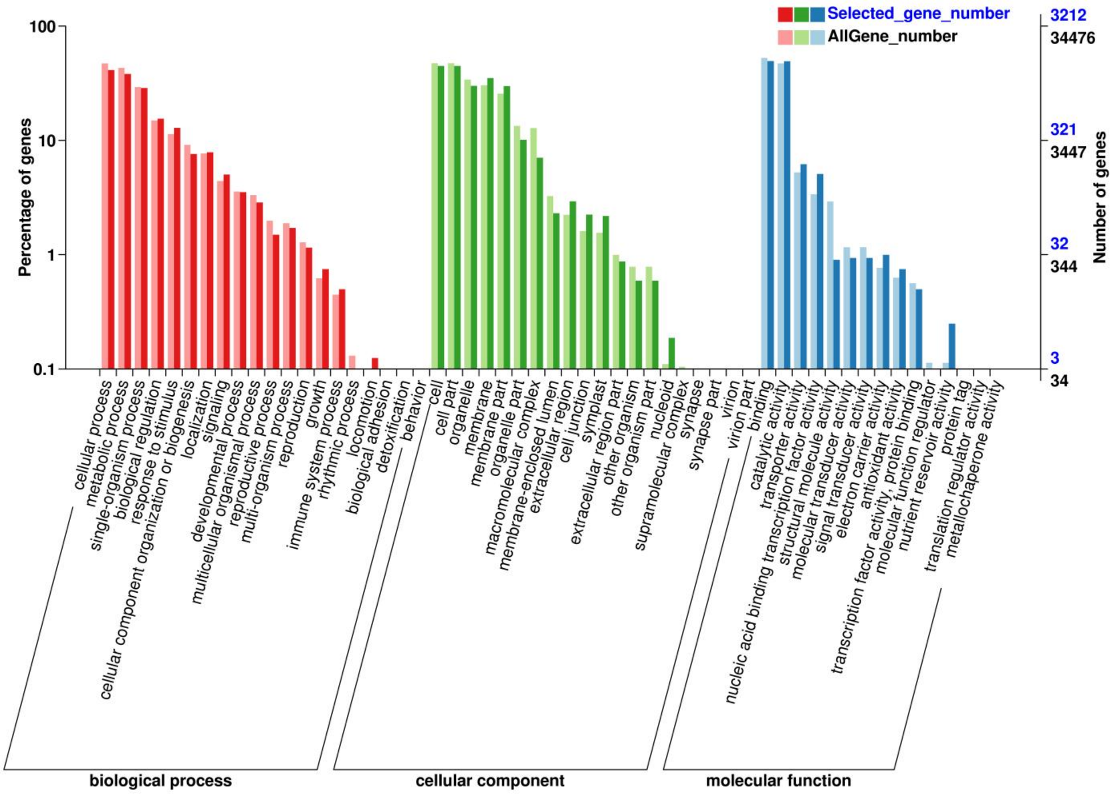

Fig. 4.11. GO class of DEGs between the 62\3 and JB231AC genotypes under 50μM Cd stress.

The result showed that 55 GO terms were grouped into three categories (biological process, cellular component, and molecular function). Binding and catalytic activity had the highest DEG number, followed by cell, cell part, cellular process, metabolic process, and so on.

To identify the metabolic pathway enrichment between 62\3 and JB231AC under 50μM Cd stress, 4764 DEGs were analyzed by KEGG. A total of 130 KEGG pathways were enriched and 20 KEGG pathways were significantly enriched (Fig. 4.12, Appendix G). Among them, plant-pathogen interaction, MAPK signaling pathway-plant, plant hormone signal transduction, galactose metabolism, and pentose and glucuronate interconversions were listed in the top five KEGG pathways.

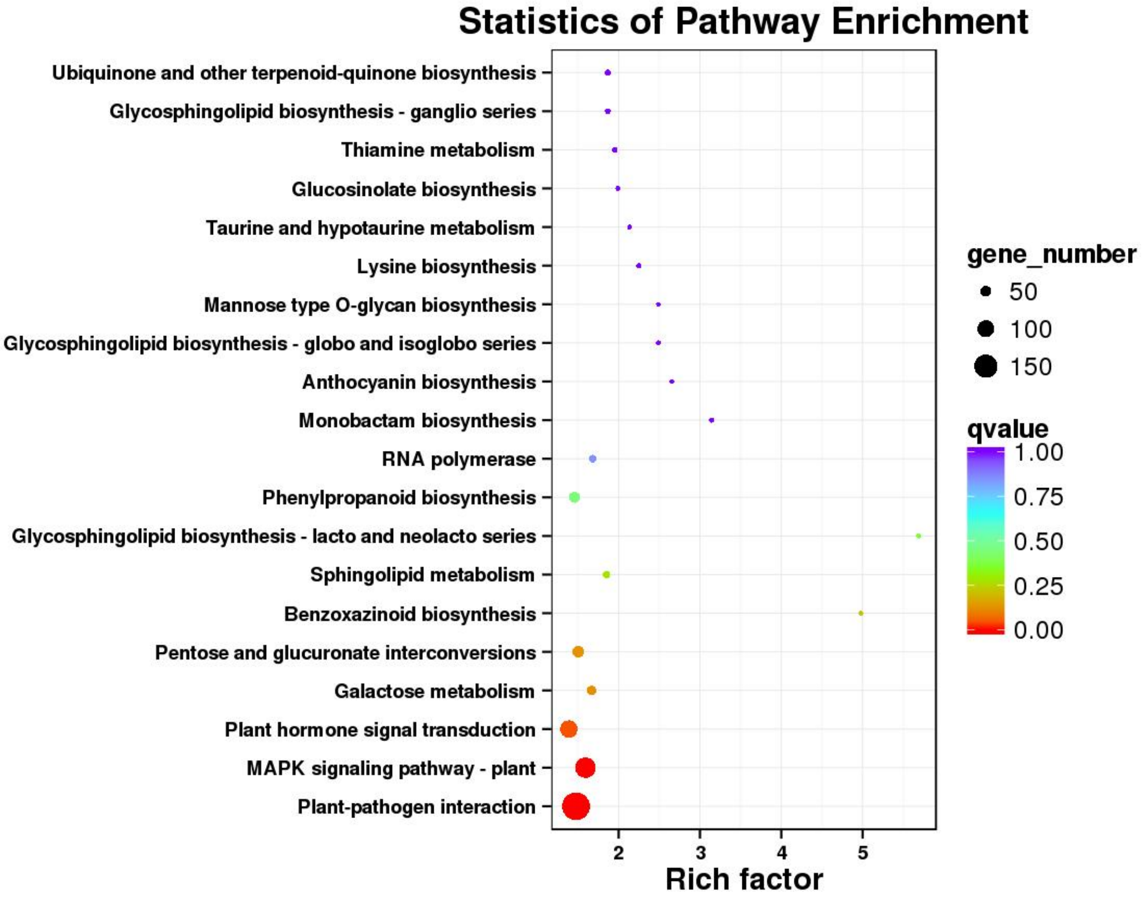

Fig. 4.12. KEGG pathways of DEGs between the 62\3 and JB231AC genotypes under 50μM Cd stress.

4.3.4 DEGs related to Cd detoxification, transportation, and accumulation

A total of 79 DEGs related to Cd detoxification, transportation, and accumulation were identified. Among them, 36 DEGs *ABC,* 24 upregulated and 12 downregulated, were identified between 62\3 and JB231AC under 50μM Cd stress, accounting for 43,4% of all selected Cd-related genes (Fig. 4.13, Appendix H). Both DEGs, *C50541. graph_c4* ($\log_2$FC = 4.987981385) and *C53176. graph_c10* ($\log_2$FC = 4,848310244), were upregulated and had a higher differential expression in 62\3 than that in JB231AC.

26 DEGs *Zn-regulated transporter, Iron-regulated transporter-like protein* (*ZIP*), 15 upregulated and 11 downregulated, were identified in 62\3 and JB231AC under 50μM Cd stress, accounting for 31.3% of all selected Cd-related genes (Fig. 4.13, Appendix I). Among them, there is the gene *c55191.graph_c0* ($\log_2$FC = 6.693782781), which was upregulated and had a higher differential expression in 62\3 than that in JB231AC.

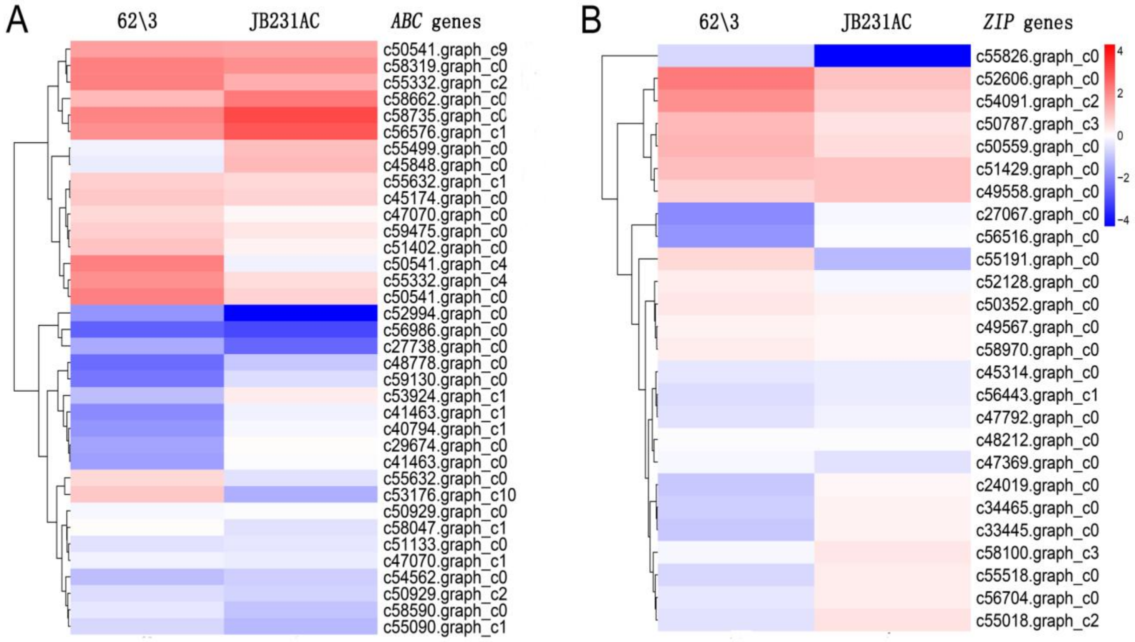

Fig. 4.13. Heat maps of DEG expression of 623 and JB231AC under 50μM Cd stress.

Moreover, DEGs Cd-related including *HIPP* (*Heavy metal associated isoprenylated plant protein*), *MTP* (*Metal tolerance protein*), *YSL* (*Metal-*

nicotianamine transporter), *HMA* (*Heavy metal ATPase*), and *NRAMP* (*Natural resistance-associated macrophage protein*) were screened out and the number was 7, 4, 3, 2, and 1, respectively (Appendix J). Among them, there were 12 upregulated and 5 downregulated with a total of 17. Upregulated gene *c37848.graph_c0* belonging to the *HIPP* family had a large differential expression level (Log_2FC = 4,28146756) and its expression level in 62\3 was higher than that in JB231AC. One upregulated *NRAMP* gene, *C53780.graph_c0* (Log_2FC = 4,207403041) was identified and had a higher expression level in 62\3. Two upregulated *HMA* genes, *c55845.graph_c0* and *c49575.graph_c0,* were screened out and both had a higher expression level in 62\3 than in JB231AC.

Conclusions to Chapter 4.

The toxic heavy metal Cd is easily absorbed and accumulated in crops and affects human health through the food chains. Two sunflower varieties of 62\3 and JB231AC were chosen as high/low Cd accumulators and physiological and transcriptomic analyses were conducted under different levels of Cd stress. The study provides important information for further research on Cd accumulation and detoxification mechanisms in sunflowers.

According to the results of the comparison of phenotype characteristics to Cd stress between high/low Cd sunflower varieties. 62\3 was sensitive to Cd toxicity, i.e., JB231AC had a higher Cd tolerance than 62\3. 62\3 was a high Cd accumulation variety and had a higher root-to-shoot Cd translocation ratio than JB231AC. In general, the genotype JB231AC had a larger biomass than 62\3 at the same concentration of Cd stress.

According to the results of the comparison of physiological responses to Cd stress in 62\3 and JB231AC. The contents of H_2O_2 and MDA were not significantly different between 62\3 and JB231AC under the same concentration of Cd stress. The activity of SOD was strongly stimulated under 50 μM Cd stress. With an increase in Cd concentration, the activity of POD in 62\3 first increased and then decreased and reached a maximum value under 25 μM Cd stresses. However, the activity of POD in JB231AC increased with an increased Cd concentration. The activity of POD was higher in JB231AC than in 62\3 and showed highly significant differences under 50 μM and 100 μM Cd stress.

According to the results, the comparison of the accumulation of trace metallic elements in the root and the aboveground part of the variety of 62\3 and JB231AC seedling was conducted. The accumulation and distribution of trace metallic elements in plants have a great relationship with species, varieties, parts, and growing environment and eventually lead to different accumulation and distribution in different parts of plants. Concerning Li, Ni, and Sr, a significant difference between the samples was mostly observed at a Cd concentration of 25 and 50 μM. The accumulation concentration of Li and Ni in the high Cd variety of 62\3 was lower than that in the low Cd variety of JB231AC. The Li and Sr concentration in the root was similar to that in the aboveground part under Cd stress, while Ni accumulated in the root of JB231AC in slightly higher concentrations than that in the aboveground part. The presence of an inverse relationship between the concentration of Cd and the concentrations of Li and Ni may indicate both the genotypic features of their transport and the presence of antagonism between the accumulation of these metals. An inverse relationship between Cd concentration and Li and Ni concentrations has been established, which may be due to genotypic features. Although, the presence of antagonism between the accumulation of these metals is not excluded.

According to the results of the identification of Cd- regulated DEGs, the two varieties had more upregulated DEGs than downregulated. Comparing 62\3 with JB231AC under 50 μM Cd stress, 4764 DEGs were identified in the comparison groups 62\3 and JB231AC, there were approximately equal numbers of up and downregulated DEGs.

According to the results of functional annotations of Cd- regulated DEGs. 55 GO terms were grouped into three categories (biological process, cellular component, and molecular function). Binding and catalytic activity had the highest DEG number, followed by cell, cell part, cellular process, and metabolic process. A total of 130

KEGG pathways were enriched and 20 KEGG pathways were significantly enriched, and plant-pathogen interaction, MAPK signaling pathway-plant, plant hormone signal transduction, galactose metabolism, pentose, and glucuronate interconversions were listed in the top five KEGG pathways.

According to the results of DEGs related to Cd detoxification, transportation, and accumulation, a total of 79 DEGs related to Cd detoxification, transportation, and accumulation were identified. Among them, 36 DEGs *ABC* and 26 DEGs *ZIP* were identified respectively. Besides, Cd-related DEGs, including *HIPP* (*Heavy metal associated isoprenylated plant protein*), *MTP* (*Metal tolerance protein*), *YSL* (*Metal-nicotianamine transporter*), *HMA*, and *NRAMP* (*Natural resistance-associated macrophage protein*), were screened out, and the number of DEGs for each was 7, 4, 3, 2, and 1, respectively.

The transcriptomic analysis showed that many *ABC* and *ZIP* genes were differentially expressed, indicating that the two kinds of genes might play a significant role in different Cd accumulation in the two varieties. Two upregulated *HMA* genes and one upregulated *NRAMP* gene were screened out.

In follow-up work, the functions of candidate genes related to Cd physiology would be further validated by the genetic transformation, and the functional molecular markers could be developed for the marker-assisted selection of sunflowers in future selection programs.

CHAPTER 5

CHARACTERISTICS OF THE CREATED SOURCE MATERIAL

5.1 Dynamics of cadmium content in the seeds

The development of a selection program for the creation of genotypes with controlled indicators of crop quality (in our case, the content of cadmium in the seeds) involves the preliminary development of a variety model. The next stage of the work is the selection of samples with initial parameter values as close as possible to the model (Table 5.1.).

Table 5.1

Dynamics of cadmium content in the seeds of inter-varietal sunflower hybrids *

№	Origin	2020		2021		X
		X	± to conditional standard	X	± to conditional standard	
Conditional standard		0,14		0,13		0,13
19/06	Rezon / PR63LLO1	0,07	-0,07	0,03	-0,1	0,05
19/32	Zorya / JG3	0,06	-0,08	0,04	-0,09	0,05
19/67	JB231AC / X51Б	0,05	-0,09	0,05	-0,08	0,05
19/40	Sumico / Snk630	0,05	-0,09	0,09	-0,04	0,07
19/41	Sumico / S2K670	0,06	-0,08	0,1	-0,03	0,08
$LSD_{0,05}$		0,02		0,02		

**-natural background of cadmium in the arable layer of the soil in the experimental areas – 0,21 mg/kg*

Currently, the indicator of cadmium content in the seeds determined in Ukraine cannot exceed 0,1 mg/kg for the confectionery sunflower and 0,2 mg/kg for the sunflower used for oil production. Taking into account the defined limits, five samples were selected, in which the cadmium content did not exceed 0,1 mg/kg during the testing period. There were samples with numbers 19/06, 19/32, 19/67, 19/40, and 19/41. In the previous chapters (Chapter 3), a high and statistically significant negative level of correlation was determined between the average values of cadmium content in the seeds and plant productivity in the group of hybrids with a low level of cadmium accumulation.

The analysis of productivity over the years of the research indicates the heterogeneity of the revealed correlation depending on environmental conditions. In a more favorable year (in terms of vegetation conditions and yield capacity) of 2020, two out of five hybrids (19/06, 19/32) had higher cadmium content in the seeds compared to 2019, which was less favorable for yield capacity formation. The same dynamics were observed in hybrids of the conditional standard.

The opposite effect was observed for the samples of 19/40 and 10/41, which in their pedigree had a common maternal form – the Sumico hybrid. The cadmium content in the seeds of the sample of 19/67 remained stable – 0,05 mg/kg with fairly significant annual fluctuations in the plant productivity index. Thus, regardless of the vegetation conditions, the cadmium content of the selected samples was statically lower than the conventional standard and was within the limits specified by the state standard.

5.2 Parameters of vegetative and generative plant development

The main criterion for the appropriateness of vegetation conditions in the northeastern Forest-steppe zone of Ukraine is the vegetation length, which is in the range of 110-120 days and corresponds to the indicators of the group of medium-early genotypes. For the convenience of evaluating this criterion in selection practice, as a rule, the indicator of the duration of the interphase period of "seedlings – flowering" is used (Table 5.2.).

The value of the duration indicator of the interphase period for the hybrids of the conditional standard was 56,7±1,8 days, which was optimal for the conditions of the zone because it ensured the maximum use of heat and moisture resources. At the same time, it allows to grow of sunflowers without desiccation since the period of technological maturation takes place at average daily temperatures of > + 14 ^{o}C.

Table 5.2

Plant development parameters of inter-varietal sunflower hybrids (2020-2021)

Traits	Conventional standard	Number of samples				
		19/06	19/32	19/67	19/40	19/41
Parameters of vegetative plant development						
“Seedling – flowering” period, days	56,7±1,8	51,7±1,5	59,2±2,1	57,5±2,0	59,1±1,3	58,6±1,7
Height, cm	163,6±9,2	128,3±8,9	180,1±12,4	193,3±11,4	133,3±5,7	160,0±9,4
Leaf number, pcs	18,4±2,1	17,6±1,8	20,3±2,8	18,6±2,1	21,6±1,7	16,4±2,3
Parameters of generative plant development						
Head diameter, cm	16,8±2,1	16,7±1,8	16,7±1,9	19±1,2	15,7±2,6	18,3±1,4
1000 seed weight, g	62,2±2,1	57,8±1,4	54,7±2,5	64,3±2,4	60,4±1,7	54,1±2,8
Seed number per head, pcs.	1106,1±56,6	655,7±43,8	802,6±73,2	911,4±67,9	682,1±52,3	1024,0±69,2
Traits of yield quality						
Seed husking, %	25,6±1,3	27,3±2,3	26,5±2,8	27,2±3,2	25,5±2,7	24,4±2,9
Oil content in seed, %	48,7±1,5	46,5±2,2	46,2±2,5	46,5±2,1	46,9±2,3	47,0±2,7

In our case, the indicators of hybrids 19/67 and 19/41 were the closest to this parameter. In terms of a steady trend toward climate warming, the level of early ripening of 19/32 and 19/40 hybrids is also acceptable. The high level of early ripening of 19/06 hybrid can be used to improve the seed productivity of plants under the scheme of individual or family selection in the future.

The number of leaves is an additional indicator that is closely correlated with the duration of vegetation. The average values of this indicator corresponded to the rating of the samples by the duration of the growing season. The range of average plant height values varied from 128,3 cm (sample 19/06) to 193,3 cm (sample 19/67), which corresponds to the technological parameters of sunflower growing and harvesting. Thus, all breeding samples selected according to the indicator of cadmium content in the seeds correspond to the optimal parameters of early maturity and are close to the morphotype of modern sunflower crop in terms of the trait set of vegetative development in the Forest-steppe zone of Ukraine.

To understand the processes of developing of new source material, indicators of actual seed productivity and 1000 seeds weight are important parameters. According to the indicators of potential seed productivity (head diameter), all the selected samples were close to or exceeded the value of the conventional standard. However, according to the indicators of the seed number (actual seed productivity), none of the selected samples exceeded the conditional standard.

A similar situation was observed for the indicator of 1000 seed weight. Only one of the selected samples (19/67) was characterized by high values of seed weight, with an average level of variation. The obtained source material also needs significant improvement in terms of yield quality indicators. The parameter values of seed huskiness and seed oil content for the conditional standard were 25,6±1,3 and 48,7±1,5%, respectively. The selected group of samples had similar but worse indicators. Moreover, the average values of all hybrids had high indicators of standard error, which indicates the varietal unevenness of the material according to these characteristics. The general analysis of the group of indicators of the gene-

generative development of plants and the yield quality of the selected hybrids indicates the need for significant selection improvement of the source material.

5.3 Plant productivity and estimated yield

The general range of the productivity index of the created hybrids was from 14,3 to 69,8 g/plant with an average value of 34,2 g. All selected samples had a higher level of plant productivity than the average for the collection.

The analysis of plant productivity depending on vegetation conditions (Table 5.3) indicated differences in plants' response to weather conditions during the years of the research. Thus, in 2020 (more favorable for the yield-forming weather conditions), only one of the 19/67 hybrids had a level of productivity close to the conventional standard. In the less favorable year of 2021, the absence of a statistically significant difference with the conditional standard was noted in samples 19/67 and 19/41.

Table 5.3

Dynamics of plant productivity of inter-varietal sunflower hybrids

№	Origin	2020		2021		X
		X	± to conditional standard	X	± to conditional standard	
Conditional standard		72,1		65,5		68,8
19/06	Rezon / PR63LLO1	43	-29,1	32,8	-32,7	37,9
19/32	Zorya / JG3	49,8	-22,3	37,9	-27,6	43,9
19/67	JB231AC / X51Б	60,8	-11,3	56,4	-9,1	58,6
19/40	Sumico / Snk630	38,8	-33,3	43,7	-21,8	41,2
19/41	Sumico / S2K670	53,8	-18,3	56,9	-8,6	55,4
$LSD_{0,05}$		12,4		11,9		

The change of dynamics in the values of inter-varietal hybrid group indicates the low current level of their selection improvement as well as the possibility of significantly increase in the productivity of plants due to the improvement of the level of ecological plasticity.

Despite the significant level of varietal differentiation of modern sunflower crop, the oil-based direction of crop utilization remains dominant. The calculated indicators of oil yield per unit area are the reflection of the dynamics of plant productivity values and the oil content in seeds (Table. 5.4).

Table 5.4

Estimated indicators of oil yield, t/ha

№	Origin	2020		2021		X
		X	± to conditional standard	X	± to conditional standard	
Conditional standard		1,94		1,75		1,84
19/06	Rezon / PR63LLO1	1,11	-0,83	0,83	-0,92	0,97
19/32	Zorya / JG3	1,30	-0,64	0,94	-0,81	1,12
19/67	JB231AC / X51Б	1,57	-0,37	1,43	-0,32	1,50
19/40	Sumico / Snk630	0,99	-0,95	1,13	-0,62	1,06
19/41	Sumico / S2K670	1,36	-0,58	1,50	-0,25	1,43
$LSD_{0,05}$		0,28		0,26		

The value as close as possible to the level of the conditional standard (1,75 t/ha) was obtained for the sample of 19/41 in 2021 – 1,50 t/ha. The difference between the indicators was 0,25 at the $LSD_{0.05}$ – 0,26 t/ha. In other cases, the values of oil yield indicators were significantly lower than the conditional standard.

5.4 Source material for the recreational use

Sunflower has an increased tendency to accumulate heavy metals, including cadmium. This is facilitated both by the features of the morphological structure of plants and the peculiarities of transport and accumulation of metal in the aboveground part. These features, as well as the dominant type of strategy in plant groups, allow us to consider the possibility of using specialized varieties in remediation programs for polluted areas.

Currently, this direction is not represented and requires the development and initial approval of the variety model with the formation of the corresponding theoretical and practical basis. Some hybrids created in the process of inter-varietal crossings clearly showed the traits of cadmium-accumulating plants. Thus, the concentration of cadmium in the sample seeds of 19/11 exceeded the level of metal concentration in the soil by five times, in sample 19/26 – by 10 times. (Table 5.5). Indicators of cadmium content were high in the aboveground parts of plants.

Table 5.5

Characteristics of created hybrids (with high-Cd uptake), 2020-2021

Selection parameters	Selection number of the hybrid		
	19/54	**19/11**	**19/26**
“Seedling-flowering” period, days	59	58	57
Height, cm	166,7	146,7	175,3
Seed productivity, g/plant	41,3	36,2	33,8
Cadmium content in seeds, mg/kg	1,1	2,1	1,16
Cadmium content in the stem, mg/kg	1,22	2,24	2,01
Removal of biologically active cadmium from the soil, g/ha*	9,25	11,34	19,9

**- calculated from a plot with an area of 12.6* m^2

The selected hybrids had the average indicators for the collection of other morpho-parameters of plants, including the weight of seeds per head. In the future, this creates prerequisites for a significant change in the habit of plants and the formation of a complex of recreational features while maintaining a sufficient level of seed productivity.

The highest level of removal of biologically active cadmium from the soil (about 20 g/ha) was noted in the sample of 19/26 obtained in combination with Polar CL X 64/3. Samples of 19/26 and 19/54 obtained with the participation of the STH-16004 line with the JG3 line and the commercial hybrid of Rezon had slightly lower indicators, namely 11,34 and 9,25 g/ha.

Conclusions to Chapter 5.

Considering the limits of cadmium content in sunflower seeds determined in Ukraine, 5 samples were selected as starting material for the next stages of selection, namely: 19/06; 19/32, 19/67, 19/40, and 19/41.

According to the results of field tests, a significant level of fluctuations of the cadmium content indicator was established depending on the weather conditions of the research years and plant productivity. The selected samples in all years of the research had statistically lower indicators of cadmium content compared to the conventional standard.

According to indicators of precociousness and vegetative development of plants, the samples correspond to the average plant morphotype for the conditions of the Forest-steppe.

The range of indicators of plant productivity of the selected samples was 37,9-58,6 g/plant, and oil content in the seeds was 46,2-47,0%, which provides estimated indicators of oil yield per unit area at the level of 0,97-1,5 t/ha.

According to the results of the field tests, three samples with signs of cadmium storage plants were selected. They are 19/54, 19/11, and 19/26. The content of cadmium in the seeds and the aboveground parts of plants exceeded the natural background by 5 or more times, which provided the possibility of removing up to 20 g/ha of biologically active cadmium from the soil.

The high level of cadmium assimilation and the significant range of vegetative and generative development of plants allow considering selected samples as source material for selection programs of soil remediation.

CONCLUSION

The dissertation theoretically summarizes and develops practical approaches to solving the scientific task of creating sunflower raw material with a controlled level of cadmium accumulation.

1. The methodology for determining the rank of selection samples of sunflower for resistance to cadmium accumulation with the following parameters of the vegetation experiment was developed and tested: analyzing background - substrate with a cadmium content of 1,0 mg/kg, the final stage of vegetation - R5.
2. A working collection based on resistance to cadmium accumulation was formed. It was established that under the conditions of the analyzing background, the content of cadmium in the above-ground part of plants varied from 0,5 to 2,6 mg/kg with an average value of 1,14 mg/kg. High resistance to cadmium accumulation in the above-ground part of plants (< 1,0 mg/kg) was noted in 10,6% of the samples. The proportion of samples with a high level of cadmium accumulation (> 2,11 mg/kg) was 6,7%.
3. According to the results of a field experiment (natural background of cadmium 0,21 mg/kg), indicators of cadmium content in seeds were determined. The average value was 0,34±0,01 with a range of variability of the indicator of 0,23 – 0,43 mg/kg. The minimum level of cadmium accumulation in seeds was noted in 16 samples, including: 64\3; JB231AC; 56\3; Neon-2011, UE0100938, UE0100018, UE0100977, S2K670, Snk630 Sumico, Polaris CL, Rezon.
4. The effectiveness of the technique for determining the rank of selection samples for resistance to cadmium accumulation was confirmed. Belonging to the corresponding group by the level of resistance to cadmium accumulation in seeds was confirmed for 63,8% of breeding samples. The highest rates of compliance were in the groups with unsatisfactory and low levels of resistance, 85,7 and 84,2%, respectively. In the group with a high level of resistance, the share of shared hybrids was 76,1%.

5. Evaluation of the working collection was carried out according to 12 indicators. The range of variation and average values according to the main selection-controlled indicators were: duration of the flowering stage period - 50 to 66 days, the average calculated value of the LAI indicator was 3,2 with a range, plant productivity values were 11,70 – 63,30 with an average of 33,6 g

6. According to the results of direct and backcrossing, it was established that when crossing "low cadmium" parental forms, only a fifth or 19% inherited the trait of resistance to cadmium accumulation by the type of heterosis. The proportion of hybrids that inherited the trait by the type of partial positive dominance and intermediate for parental forms accounted for 9,5% each. In the vast majority of cases, 62% of trait inheritance occurred according to the type of depression and partial negative dominance. In the crossbreeding variants in the group of "high cadmium" samples and in inter-group crossbreeding, depression and partial negative dominance were also the predominant type of inheritance. The total share of these types of phenotypic inheritance was 85,7% when crossing "high cadmium" samples and 66,7% in inter-group crossings.

7. According to the results of crossing, the range of variability of the indicator of cadmium content in seeds was expanded, which was 0,05 – 2,24 with the average value for hybrids of 0,41 mg/kg. The largest share of hybrids with minimal cadmium content (greater than 0,1) was obtained in combinations using as a maternal component the 56\3 line, commercial hybrids Rezon and Sumico and as a parent component (pollinator) - lines JB231AC , X51B, as well as commercial hybrid Sumico.

8. In the first and second hybrid generations, the indicators of the correlation structure of the selectively controlled traits were optimized. The indicator of cadmium content in seeds had a statistically significant negative correlation with the indicator of plant productivity (r = - 0,66) and a positive correlation with the LAR coefficient (r = 0,55).

9. According to the results of the comparison of physiological responses to Cd stress in 62\3 and JB231AC. The contents of H_2O_2 and MDA were not

significantly different between 62\3 and JB231AC under the same concentration of Cd stress. The activity of SOD was strongly stimulated under 50 µM Cd stress. With an increase in Cd concentration, the activity of POD in 62\3 first increased and then decreased and reached a maximum value under 25 µM Cd stresses. However, the activity of POD in JB231AC increased with an increased of Cd concentration. The activity of POD was higher in JB231AC than in 62\3 and showed highly significant differences under 50 µM and 100 µM Cd stress.

10. Concerning Li, Ni, and Sr, a significant difference between the samples was mostly observed at a Cd concentration of 25 and 50 µM. The accumulation concentration of Li and Ni in the high Cd variety of 62\3 was lower than that in the low Cd variety of JB231AC. The Li and Sr concentration in the root was similar to that in the aboveground part under Cd stress, while Ni accumulated in the root of JB231AC in slightly higher concentrations than that in the aboveground part. The presence of an inverse relationship between the concentration of Cd and the concentrations of Li and Ni may indicate both the genotypic features of their transport and the presence of antagonism between the accumulation of these metals. An inverse relationship between Cd concentration and Li and Ni concentrations has been established, which may be due to genotypic features. Although, the presence of antagonism between the accumulation of these metals is not excluded.

11. According to the results of DEGs related to Cd detoxification, transportation, and accumulation, a total of 79 DEGs related to Cd detoxification, transportation, and accumulation were identified. Among them, 36 DEGs *ABC* and 26 DEGs *ZIP* were identified respectively. Besides, Cd-related DEGs, including *HIPP* (*Heavy metal associated isoprenylated plant protein*), *MTP* (*Metal tolerance protein*), *YSL* (*Metal-nicotianamine transporter*), *HMA*, and *NRAMP* (*Natural resistance-associated macrophage protein*), were screened out, and the number of DEGs for each was 7, 4, 3, 2, and 1, respectively. The transcriptomic analysis showed that many *ABC* and *ZIP* genes were differentially expressed, indicating that the two kinds of genes might play a significant role in different Cd accumulation in

the two varieties. Two upregulated *HMA* genes and one upregulated *NRAMP* gene were screened out.

12. According to the results of field tests, 5 hybrid samples were selected: 19/06; 19/32, 19/67, 19/40 and 19/41. The maximum level of cadmium content in the seeds of the samples was less than 0,1 mg/kg and statistically significantly exceeded the value of the indicator of the conditional standard. The range of indicators of plant productivity was 37,9-58,6 g/plant, productivity 2,1-3,2 t/ha, oil content in seeds 46,2-4,0%, which provides calculated indicators of oil yield at the level of 0,94-1,48 t/ha.

13. Three samples (19/54, 19/11 and 19/26) with signs of plant species of cadmium accumulators were selected. The high level of cadmium concentration in the above-ground part of plants (5 or more times higher than the background values) allows us to consider the selected samples as the starting material for creating phytoremedial genotypes. The estimated level of removal of biologically active cadmium from the soil is 20,0 g/ha

Recommendations for selection practice

- in developing of linear sunflower material, its preliminary testing for resistance to cadmium accumulation should to carry out using an analytical background with a cadmium concentration of 1,0 mg/kg;
- in creating of sunflower source material with a minimum level of cadmium accumulation, selection sample of JB231AC and intervarietal hybrids of 19/06, 19/32 , 19/67, 19/40 , 19/41 can be used;
- sample of 62/3 and intervarietal hybrids of 19/54 H, UCh 19/11, 19/26 should be used in selection programs of the phytoremedial direction.

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APPENDICES

Appendix A

Helianthus annuus L

Theme	Створення вихідного матеріалу зернових та олійних культур, стійких до накопичення важких металів	Creation of the initial material of grains and oil crops, resistant to the accumulation of heavy metals
Scientific supervisor	Троценко В.	Trotsenko V.
PhD student	Фу Юаньчжи	Fu Yuanzhi
Date of experiment start	02\07\11\16. 03. 2019.	

Vegetative experiment for evaluating the resistance of breeding samples to cadmium accumulation. Substrate with a cadmium content of 1.0 mg/kg (March, 2019)

Appendix B

A field experiment on the study of a working sunflower collection for resistance to cadmium accumulation. Natural background of cadmium in soil 0.21 mg/kg (Stage R-4 - R-6, July, 2021)

Appendix C

The process of intervarietal hybridization, in the photo on the right is a breeding sample UE0100977 (Stage R-5.5. July, 2019)

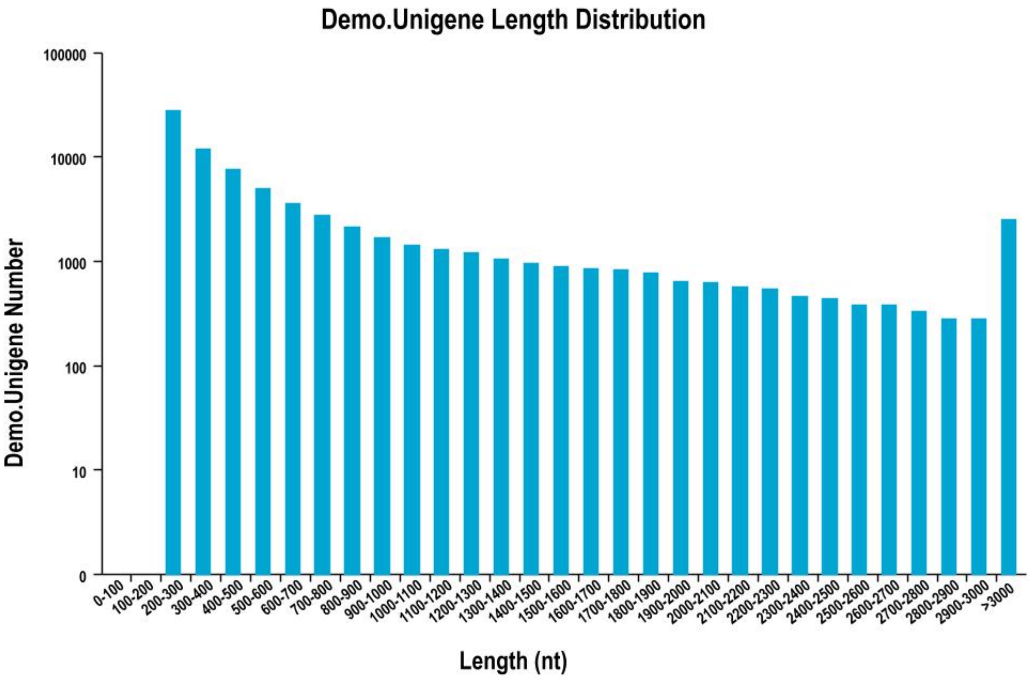
Appendix D

Line 62\3 (photo left, 33) stage R-5.3 and line JB231AC (51) stage R-5.5. (July, 2019)

Appendix E

Vegetation of lines 62\3 and JB231AC under normal conditions (A) and under conditions of cadmium stress (B), (Stage V-6, 2020)

Appendix F



Length distribution of unigenes

Appendix G

All KGEE enrichment pathways of DEGs between 62\3 and JB231AC under 50µM Cd stress

#Kegg_pathway	ko_id	P-value	Corrected_P-value	rich_factor		
Plant-pathogen interaction	ko04626	1.49E-08	1.94E-06	1.476238498		
MAPK signaling pathway - plant	ko04016	4.82E-08	6.26E-06	1.592552301		
Plant hormone signal transduction	ko04075	0.00032245	0.041918467	1.387724551		
Galactose metabolism	ko00052	0.000971955	0.126354202	1.66655316		
Pentose and glucuronate interconversions	ko00040	0.000999653	0.129954886	1.503859469		
Benzoxazinoid biosynthesis	ko00402	0.001658844	0.215649684	4.978293413		
Sphingolipid metabolism	ko00600	0.002129854	0.276880972	1.852388247		
Glycosphingolipid biosynthesis - lacto and neolacto series	ko00601	0.002763461	0.359249931	5.689478186		
Phenylpropanoid biosynthesis	ko00940	0.003430164	0.445921368	1.45842962		
RNA polymerase	ko03020	0.006515125	0.846966193	1.681855883		
Monobactam biosynthesis	ko00261	0.008724463	1	3.144185314		
Anthocyanin biosynthesis	ko00942	0.056202105	1	2.65508982		
Glycosphingolipid biosynthesis - globo and isoglobo series	ko00603	0.043747133	1	2.489146707		
Mannose type O-glycan biosynthesis	ko00515	0.345203507	1	2.489146707		

Lysine biosynthesis	ko00300	0.031040694	1	2.248261541		
Taurine and hypotaurine metabolism	ko00430	0.159766856	1	2.13355432		
Glucosinolate biosynthesis	ko00966	0.134374994	1	1.991317365		
Thiamine metabolism	ko00730	0.028360094	1	1.952271927		
Glycosphingolipid biosynthesis - ganglio series	ko00604	0.047069164	1	1.86686003		
Ubiquinone and other terpenoid-quinone biosynthesis	ko00130	0.01256592	1	1.86686003		
Arachidonic acid metabolism	ko00590	0.024060232	1	1.86686003		
Limonene and pinene degradation	ko00903	0.296866058	1	1.572092657		
Glycosaminoglycan degradation	ko00531	0.088798829	1	1.564606501		
Pantothenate and CoA biosynthesis	ko00770	0.081835195	1	1.551675869		
Ascorbate and aldarate metabolism	ko00053	0.010271023	1	1.535592993		
Zeatin biosynthesis	ko00908	0.043605886	1	1.513401198		
Monoterpenoid biosynthesis	ko00902	0.232846385	1	1.508573762		
ABC transporters	ko02010	0.012500241	1	1.50288103		
Cutin, suberine and wax biosynthesis	ko00073	0.111568957	1	1.500307604		
Valine, leucine and isoleucine biosynthesis	ko00290	0.20881019	1	1.493488024		
Other glycan degradation	ko00511	0.068120277	1	1.488835413		

Diterpenoid biosynthesis	ko00904	0.08614999	1	1.48289591		
Steroid biosynthesis	ko00100	0.183042396	1	1.448230811		
beta-Alanine metabolism	ko00410	0.094005182	1	1.422369547		
C5-Branched dibasic acid metabolism	ko00660	0.417669481	1	1.422369547		
Betalain biosynthesis	ko00965	0.30821317	1	1.422369547		
Carotenoid biosynthesis	ko00906	0.15127323	1	1.389291185		
Brassinosteroid biosynthesis	ko00905	0.292235167	1	1.382859281		
Glycine, serine and threonine metabolism	ko00260	0.083983245	1	1.360527392		
Glycerolipid metabolism	ko00561	0.062101294	1	1.357716385		
Alanine, aspartate and glutamate metabolism	ko00250	0.136856979	1	1.357716385		
Sulfur metabolism	ko00920	0.251834158	1	1.32754491		
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	ko00563	0.313809526	1	1.298685238		
2-Oxocarboxylic acid metabolism	ko01210	0.232371142	1	1.25665659		
Butanoate metabolism	ko00650	0.374548112	1	1.244573353		
Biosynthesis of unsaturated fatty acids	ko01040	0.311817899	1	1.244573353		
Aminoacyl-tRNA biosynthesis	ko00970	0.227695326	1	1.177133418		
Flavone and flavonol biosynthesis	ko00944	0.52078052	1	1.171363156		

Phosphonate and phosphinate metabolism	ko00440	0.52078052	1	1.171363156		
Cyanoamino acid metabolism	ko00460	0.333729728	1	1.155675257		
Lysine degradation	ko00310	0.390093491	1	1.154386878		
Inositol phosphate metabolism	ko00562	0.302533448	1	1.148836942		
Starch and sucrose metabolism	ko00500	0.170808488	1	1.143662		
Valine, leucine and isoleucine degradation	ko00280	0.369564725	1	1.125527206		
Amino sugar and nucleotide sugar metabolism	ko00520	0.259412097	1	1.112467802		
Lipoic acid metabolism	ko00785	0.61438208	1	1.106287425		
Other types of O-glycan biosynthesis	ko00514	0.518626903	1	1.106287425		
Linoleic acid metabolism	ko00591	0.448925685	1	1.106287425		
Isoquinoline alkaloid biosynthesis	ko00950	0.453235723	1	1.091132803		
Nicotinate and nicotinamide metabolism	ko00760	0.498062983	1	1.06677716		
Fructose and mannose metabolism	ko00051	0.445091981	1	1.064062714		
Ether lipid metabolism	ko00565	0.462370663	1	1.063324807		
Basal transcription factors	ko03022	0.499043218	1	1.056001633		
Folate biosynthesis	ko00790	0.499860465	1	1.048061771		
Tropane, piperidine and pyridine alkaloid biosynthesis	ko00960	0.515844666	1	1.048061771		

Stilbenoid, diarylheptanoid and gingerol biosynthesis	ko00945	0.501184935	1	1.037144461		
Fatty acid elongation	ko00062	0.514943212	1	1.029991741		
Flavonoid biosynthesis	ko00941	0.493173589	1	1.027776705		
Sesquiterpenoid and triterpenoid biosynthesis	ko00909	0.545222133	1	1.008261957		
Homologous recombination	ko03440	0.525305254	1	1.008261957		
Protein export	ko03060	0.580917325	1	0.976135963		
Glutathione metabolism	ko00480	0.585594235	1	0.972769977		
Biosynthesis of amino acids	ko01230	0.721062192	1	0.932835095		
Selenocompound metabolism	ko00450	0.639102185	1	0.926194123		
Histidine metabolism	ko00340	0.657333476	1	0.905144257		
Ubiquitin mediated proteolysis	ko04120	0.746510885	1	0.902214988		
Endocytosis	ko04144	0.813614573	1	0.894760193		
Glycolysis / Gluconeogenesis	ko00010	0.776700781	1	0.892064138		
Peroxisome	ko04146	0.736354154	1	0.886188356		
Porphyrin and chlorophyll metabolism	ko00860	0.712226494	1	0.883245606		
Arginine biosynthesis	ko00220	0.69033058	1	0.873384809		
Riboflavin metabolism	ko00740	0.691895177	1	0.865790159		

Phosphatidylinositol signaling system	ko04070	0.770743316	1	0.858326451		
N-Glycan biosynthesis	ko00510	0.728753562	1	0.856480587		
Isoflavonoid biosynthesis	ko00943	0.697036048	1	0.853421728		
Glyoxylate and dicarboxylate metabolism	ko00630	0.800159289	1	0.84630988		
SNARE interactions in vesicular transport	ko04130	0.719442623	1	0.843778545		
RNA degradation	ko03018	0.842740865	1	0.843778545		
Nitrogen metabolism	ko00910	0.730268765	1	0.841401704		
Sulfur relay system	ko04122	0.719363647	1	0.829715569		
alpha-Linolenic acid metabolism	ko00592	0.80092795	1	0.817331754		
Tryptophan metabolism	ko00380	0.808571109	1	0.811277445		
Fatty acid metabolism	ko01212	0.832173129	1	0.810419858		
Phenylalanine metabolism	ko00360	0.796173333	1	0.807290824		
Glycerophospholipid metabolism	ko00564	0.873712361	1	0.806201362		
Arginine and proline metabolism	ko00330	0.810353539	1	0.802950551		
Fatty acid degradation	ko00071	0.804642733	1	0.80008287		
Cysteine and methionine metabolism	ko00270	0.882011196	1	0.79972585		
Carbon metabolism	ko01200	0.972787905	1	0.796526946		
Carbon fixation in photosynthetic organisms	ko00710	0.894634653	1	0.784959127		
Propanoate metabolism	ko00640	0.816253599	1	0.780908771		

Mismatch repair	ko03430	0.812024387	1	0.774401198		
DNA replication	ko03030	0.84998352	1	0.75940069		
Pyrimidine metabolism	ko00240	0.878729007	1	0.750153802		
Phenylalanine, tyrosine and tryptophan biosynthesis	ko00400	0.835908421	1	0.73752495		
Tyrosine metabolism	ko00350	0.875281472	1	0.734502307		
Base excision repair	ko03410	0.852437815	1	0.719753265		
Pyruvate metabolism	ko00620	0.963026251	1	0.693696623		
Purine metabolism	ko00230	0.959568063	1	0.68508625		
Photosynthesis	ko00195	0.965051921	1	0.6669484		
Photosynthesis - antenna proteins	ko00196	0.86549459	1	0.663772455		
Spliceosome	ko03040	0.998383617	1	0.661389071		
Protein processing in endoplasmic reticulum	ko04141	0.999764119	1	0.632642431		
Circadian rhythm - plant	ko04712	0.962389294	1	0.630163723		
Fatty acid biosynthesis	ko00061	0.933547272	1	0.61587135		
Biotin metabolism	ko00780	0.857864555	1	0.603429505		
Autophagy - other	ko04136	0.909166965	1	0.603429505		
Terpenoid backbone biosynthesis	ko00900	0.94112168	1	0.603429505		
Ribosome biogenesis in eukaryotes	ko03008	0.981696286	1	0.595230734		

RNA transport	ko03013	0.999776117	1	0.562763603		
Nucleotide excision repair	ko03420	0.97483588	1	0.553143713		
Pentose phosphate pathway	ko00030	0.992219073	1	0.484000749		
Proteasome	ko03050	0.9939	1	0.449169331		
Phagosome	ko04145	0.999003051	1	0.441425032		
Various types of N-glycan biosynthesis	ko00513	0.972591441	1	0.439261184		
Vitamin B6 metabolism	ko00750	0.929253267	1	0.398263473		
Ribosome	ko03010	1	1	0.350584043		
Oxidative phosphorylation	ko00190	0.999997588	1	0.346590048		
mRNA surveillance pathway	ko03015	0.99998906	1	0.339429096		
Citrate cycle (TCA cycle)	ko00020	0.999425242	1	0.304017918		

Appendix H

Details of DEGs predicted to belong to the ATP-binding cassette (ABC) transporter family

#ID	J-50-1_FPKM	J-50-2_FPKM	J-50-3_FPKM	S-50-1_FPKM	S-50-2_FPKM	S-50-3_FPKM	FDR	log$_2$FC	regulated
c50541.graph_c4	3.72	3.3	4.11	154.09	138.78	141.25	1.23E-263	4.98798139	up
c53176.graph_c10	0.36	1	1.22	43.52	36.62	40.57	1.44E-49	4.84831024	up
c52994.graph_c0	0	0	0.05	2.8	1.4	1.86	7.86E-11	3.81518313	up
c50541.graph_c0	12.83	13.73	14.9	150.01	144.46	155.18	0	3.43975842	up
c27738.graph_c0	0.13	0.13	0.26	3.24	2.3	3.27	2.62E-12	3.1697114	up
c55332.graph_c4	11.6	12.02	9.06	108.93	106.22	115.19	3.44E-252	3.0863968	up
c58590.graph_c0	1.49	0.97	1.49	9.34	9.47	9.8	1.69E-72	2.84954158	up
c55632.graph_c0	2.15	2.28	3.27	30.62	29.66	33.19	1.68E-10	2.78648771	up
c51402.graph_c0	6.86	7.49	6.54	47.45	45.56	44.52	2.96E-111	2.5874737	up
c56986.graph_c0	0.1	0	0.17	0.88	0.59	0.94	6.36E-07	2.36264154	up
c47070.graph_c0	7.31	4.71	5.27	30.53	29.94	29.79	3.32E-194	2.29912211	up
c55332.graph_c2	30.36	28.91	25.88	147.18	140.29	144.85	0	2.15138921	up
c59475.graph_c0	6.13	9.31	9.61	34.18	44.31	32.08	4.06E-13	1.76563179	up
c55090.graph_c1	1.54	0.66	1.21	8.83	5.94	6.42	2.01E-11	1.74322985	up
c54562.graph_c0	2.29	1.74	1.2	4.57	4.76	4.53	9.07E-19	1.5002142	up
c50929.graph_c2	0.94	0.98	3.67	6.66	8.55	8.65	0.00238194	1.44498806	up
c58319.graph_c0	57.56	62.98	49.09	152.2	151.64	150.97	6.94E-126	1.34480369	up

c58047.graph_c1	2.58	2.72	2.5	11.89	16.96	15.09	5.12E-26	1.31453582	up
c47070.graph_c1	4.95	2.99	2.73	10.26	9.57	13.5	0.00039341	1.27033878	up
c45174.graph_c0	15.47	14.13	11.23	41.15	36.08	39.59	3.62E-17	1.24555942	up
c51133.graph_c0	3.04	3.17	2.93	9	7.88	8.93	2.64E-18	1.2322318	up
c55632.graph_c1	12.02	10.43	12.89	37.86	36.97	38.58	1.79E-43	1.17369108	up
c50541.graph_c9	41.55	35.89	30	80.71	75.29	88.89	2.21E-26	1.16789881	up
c50929.graph_c0	3.95	5.8	5.52	12.22	14.37	12.71	1.32E-15	1.08379177	up
c53924.graph_c1	6.99	9.27	7.55	4.28	4.25	4.17	2.46E-14	-1.0711859	down
c55499.graph_c0	16.06	20.97	23.31	11.57	10.69	10.89	7.06E-19	-1.0716857	down
c58662.graph_c0	92.23	108.35	96.48	53.45	52.3	54	1.87E-139	-1.1295243	down
c48778.graph_c0	1.37	1.11	1.98	0.88	1.09	0.85	2.81E-05	-1.1804992	down
c41463.graph_c0	4.55	4.98	5.19	2.51	2.63	2.48	8.44E-14	-1.1884745	down
c40794.graph_c1	3.3	4.8	5.2	2	2.22	2.13	0.0042429	-1.2128139	down
c59130.graph_c0	2.03	2.41	2.57	1.45	1.23	0.77	7.40E-06	-1.2259088	down
c56576.graph_c1	210.28	234.94	218.55	109.39	107.84	110.66	7.60E-253	-1.2361305	down
c29674.graph_c0	5.33	6.06	5.12	2.5	2.68	2.75	4.75E-31	-1.2935627	down
c41463.graph_c1	3.74	4.02	3.41	2.06	1.28	1.6	3.39E-09	-1.3842203	down
c58735.graph_c0	302.26	337.35	284.01	133.44	130.34	139.38	4.13E-241	-1.4411894	down
c45848.graph_c0	23.56	21.93	27.91	11.05	11.51	8.88	2.73E-07	-1.4769049	down

Appendix I

Details of DEGs predicted to belong to the Zn-regulated transporter, Iron-regulated transporter-like Protein (ZIP) family

#ID	J-50-1_FPKM	J-50-2_FPKM	J-50-3_FPKM	S-50-1_FPKM	S-50-2_FPKM	S-50-3_FPKM	FDR	$\log_2$FC	regulated
c56443.graph_c1	0.77	1.04	1.87	3.88	4.7	3.06	5.59E-27	2.72735	up
c24019.graph_c0	6.76	3.67	3.24	2.82	2.23	2.27	0.00277	-1.0723	down
c56704.graph_c0	9.77	8.79	10.6	5.1	3.78	6.91	9.14E-08	-1.0386	down
c55826.graph_c0	0	0	0	2.07	4.04	3.04	1.18E-14	4.4988	up
c51429.graph_c0	97.26	99.94	97.44	47.09	45.87	45.76	#######	-1.2482	down
c52606.graph_c0	90.4	91.55	90.65	282	264.73	268.19	#######	1.30322	up
c45314.graph_c0	0.82	1.71	1.49	5.45	4.29	6.25	0.00087	1.48556	up
c49558.graph_c0	105.43	111.94	113.17	26.99	25.57	26.8	#######	-2.1904	down
c27067.graph_c0	1.77	1.73	2.16	0.72	0.26	0.68	9.67E-06	-1.8284	down
c48212.graph_c0	1.88	3.53	3.47	8.6	9.48	8.02	1.91E-06	1.20852	up
c54091.graph_c2	53.03	54.33	53.36	154.04	152.07	155.21	#######	1.22178	up
c58100.graph_c3	13.82	16.72	13.43	6.82	7.13	7.09	2.11E-15	-1.0064	down

c56516.graph_c0	2.48	3.35	2.41	0.93	0.54	0.66	1.71E-16	-2.1202	down
c52128.graph_c0	2.34	2.31	1.67	13.67	12.69	15.97	1.01E-08	1.3931	up
c55018.graph_c2	16.73	17.47	16.33	5.72	4.35	4.87	5.18E-62	-2.2832	down
c49567.graph_c0	4.99	4.25	4.31	10.64	10.55	13.02	4.26E-19	1.06355	up
c58970.graph_c0	5.02	5.32	5.19	13.98	12.72	15.23	1.08E-12	1.16076	up
c34465.graph_c0	7.48	6.47	8.27	2.63	2.62	2.47	1.26E-13	-1.7359	down
c47792.graph_c0	1.04	1.97	1.29	4.77	4	4.41	3.04E-06	1.32203	up
c55518.graph_c0	8.25	7.81	9.49	4.22	2.53	4.51	7.54E-05	-1.3814	down
c50787.graph_c3	15.28	14.96	15.05	57.87	52.19	51.33	8.13E-60	1.58354	up
c55191.graph_c0	0.08	0	0.09	21.96	24.75	23.3	8.27E-66	6.69378	up
c47369.graph_c0	0.41	1.56	0.18	7.05	7.91	7.91	1.12E-05	2.48661	up
c50352.graph_c0	6.99	6.76	5.73	14.48	16.42	17.1	1.20E-14	1.11135	up
c50559.graph_c0	24.49	27.82	22.72	60.88	63.39	60.22	2.52E-37	1.04447	up
c33445.graph_c0	5.59	6.12	6.57	3.81	2.42	2.31	1.36E-07	-1.2627	down
J: JB231AC; S: 62\3									

Appendix J

Details of differentially-expressed genes (DEGs) predicted to the other Cd-related gene

Gene name	#ID	J-50-1_	J-50-2_	J-50-3_	S-50-1_FPKM	S-50-2_FPKM	S-50-3_FPKM	FDR	$\log_2$FC	regulated
		FPKM	FPKM	FPKM						
HIPP	c32791.graph_c0	2.49	4.21	3.41	62.32	67.14	70.33	1.82E-72	3.958016688	up
	c35218.graph_c0	6.51	5.36	6.2	47.89	33.58	39.38	1.66E-38	2.3814266	up
	c24676.graph_c0	411.97	438.13	454.87	129.26	121.39	123.99	0	-2.052998308	down
	c42786.graph_c0	0	0	0	6.24	4.67	4.89	3.52E-11	3.999708317	up
	c59325.graph_c0	15.03	14.69	14.59	3	6.03	5.1	5.86E-08	-1.808214168	down
	c37848.graph_c0	8.57	5.44	8.71	189.02	183.28	189.88	1.91E-206	4.281467565	up
	c54459.graph_c0	94.27	98.74	92.57	50.58	51.28	49.34	1.12E-105	-1.23736363	down
MTP	c37740.graph_c1	6.54	5.5	4.95	22.8	21.9	22.72	1.79E-23	1.707868125	up
	c49045.graph_c0	1.63	1.24	1.14	3.68	3.16	4.78	3.04E-05	1.07476188	up
	c46037.graph_c0	326.47	303.6	299.36	176.24	189.71	168.07	9.77E-109	-1.25152971	down
	c37740.graph_c0	5.28	4.04	3.37	17.78	14.99	16.95	4.83E-18	1.688346683	up

YSL	c54988.graph_c0	161.51	155.81	164.78	37.29	38.43	38.15	8.55E-264	-1.716382004	down
	c44241.graph_c0	13.95	13.29	12.93	56.69	55.96	56.4	1.79E-178	1.830158148	up
	c43401.graph_c0	0.87	0.98	0.75	2.14	2.15	2.08	0.00011022	1.015036548	up
HMA	c55845.graph_c0	9.61	7.73	6.73	33.67	36.03	35.28	1.46E-137	1.999366366	up
	c49575.graph_c0	2.16	1.75	1.47	5.54	6.89	5.77	1.21E-19	1.472413229	up
NRAMP	c53780.graph_c0	0.23	0.39	0.25	14.01	9.13	14.84	4.24E-28	4.207403041	up

HIPP：Heavy metal associated isoprenylated plant proteins，MTP：Metal tolerance protein gene，YSL：Metal-nicotianamine transporter，HMA：Heavy metal ATPase，NRAMP：Natural resistance-associated macrophage protein

ЗАТВЕРДЖУЮ
Проректор з науково-педагогічної
та навчальної роботи
д. б. н., професор
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ДОВІДКА

про впровадження результатів наукових досліджень у навчальному процесі

Видана **Фу Юаньчжі** у тому, що матеріали дисертаційної роботи «Селекція соняшику на стійкість до накопичення кадмію», які опубліковані у статтях:

- **Fu Yuanzhi**, Wu Liuliu, Trotsenko V., Zhatova H. Screening of variety collections of sunflower and winter wheat for Cadmium low accumulation.- Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology, 3 (37), 2019. – . 42-47. DOI https://doi.org/10.32845/agrobio.2019.37
- **Fu Yuanzhi,** Trotsenko Volodymyr. Accumulation of heavy metals in sunflower seedlings under the influence of cadmium stress. Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology, 3 (45), 2021. C. – 64-70. DOI https://doi.org/10.32845/agrobio.2021.3.8
- **Fu Yuanzhi,** Trotsenko Volodymyr. Ways of the cadmium accumulation monitoring in sunflower and other crops: overview. - Bulletin of Sumy National Agrarian University. The series "Agronomy and Biology, 4 (46), 2021. C. – 89-96. DOI https://doi.org/10.32845/agrobio.2021.4.13
- **Fu Yuanzhi**, Zhatova Halyna, Li Yuqing, Liu Qiao, Trotsenko Volodymyr, Li Chengqi. Physiological and Transcriptomic Comparison of Two Sunflower (Helianthus annuus L.) Cultivars With High/Low Cadmium Accumulation [J]. Frontiers in Plant Science, 2022, 13. DOI: 10.3389/FPLS.2022.854386

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

Довідка видана для подання до спеціалізованої вченої ради

Завідувач кафедри
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ЗАТВЕРДЖУЮ
Заступник директора з наукової роботи
………………………… М. Г. Собко
07.09.2022 р.

00724927

А К Т

передачі зразків насіння міжсортових гібридів соняшнику до лабораторії селекції і насінництва Інституту СГПС НААН.

Для продовження селекційної роботи передаються міжсортові гібриди соняшнику створені за участю аспірантки кафедри агротехнологій та ґрунтознавства Сумського НАУ **Фу Юаньчжі** в процесі виконання дисертаційної роботи «**Селекція соняшнику стійкого до накопичення кадмію**»

Номер реєстрації	Походження			Вміст кадмію у насінні, мг\кг*
	материнська форма	/	запилювач	
19/01	*64/3*	/	*56/3*	0,48
19/02	*64/3*	/	*Rezon*	0,16
19/03	*64/3*	/	*Х51Б*	0,27
19/04	*64/3*	/	*Чк.гігант*	0,65
19/05	*56/3*	/	*64/3*	0,09
19/06	*Rezon*	/	*PR63LLO1*	0,05
19/07	*56/3*	/	*Rezon*	0,28
19/08	*56/3*	/	*Х51Б*	0,16
19/09	*56/3*	/	*Чк.гігант*	0,36
19/10	*56/3*	/	*Sumico*	0,1
19/11	*Polar CL*	/	*64/3*	2,1
19/12	*Polar CL*	/	*56/3*	0,4
19/13	*Polar CL*	/	*Х51Б*	0,18
19/14	*Polar CL*	/	*Чк.гігант*	0,37
19/15	*Polar CL*	/	*Sumico*	0,09
19/16	*Rezon*	/	*56/3*	0,09
19/17	*Rezon*	/	*Polar CL*	0,21

Номер реєстрації	Походження			Вміст кадмію у насінні, мг\кг*
	материнська форма	/	запилювач	
19/36	*Чк.гігант*	/	*Слобож*	0,47
19/37	*Sumico*	/	*56/3*	0,43
19/38	*Sumico*	/	*Polar CL*	0,11
19/39	*Sumico*	/	*Rezon*	0,14
19/40	*Sumico*	/	*Snk630*	0,07
19/41	*Sumico*	/	*S2K670*	0,08
19/42	*Sumico*	/	*Х51Б*	0,1
19/43	*Sumico*	/	*Чк.гігант*	0,25
19/44	*PR63LLO1*	/	*Rezon*	0,47
19/45	*62/3*	/	*PR63LLO1*	1
19/46	*62/3*	/	*JG3*	0,78
19/47	*JG3*	/	*62/3*	0,56
19/48	*JG3*	/	*PR63LLO1*	1,1
19/49	*Зоря*	/	*62/3*	0,81
19/50	*Зоря*	/	*PR63LLO1*	0,44
19/51	*715 (США)*	/	*PR63LLO1*	0,67
19/52	*715 (США)*	/	*Слобож*	0,53

19/18	Rezon / S2K670	0,21
19/19	Rezon / X51Б	0,18
19/20	Rezon / Sumico	0,09
19/21	Snk630 / Polar CL	0,23
19/22	Snk630 / Rezon	0,24
19/23	JB231AC / 64/3	0,44
19/24	JB231AC / 56/3	0,16
19/25	JB231AC / Polar CL	0,66
19/26	STH-16004 / Rezon	1,16
19/27	S2K670 / 56/3	0,19
19/28	S2K670 / Polar CL	0,2
19/29	S2K670 / X51Б	0,18
19/30	S2K670 / Чк.гігант	0,37
19/31	S2K670 / Sumico	0,1
19/32	Зоря / JG3	0,05
19/32	X51Б / Слобож	0,36
19/33	Чк.гігант / 56/3	0,36
19/34	Чк.гігант / Polar CL	0,55
19/35	Чк.гігант / Rezon	0,38
19/53	715 (США) / Sumico	0,34
19/54	STH-16004 / JG3	1,1
19/55	STH-16004 / 712 (США)	0,59
19/57	56/3 / Polar CL	0,09
19/63	Слобож / Sumico	0,08
19/64	64/3 / Sumico	0,14
19/66	JB231AC / Snk630	0,19
19/67	JB231AC / X51Б	0,05
19/69	Зоря / 715 (США)	0,67
19/70	62/3 / Зоря	0,67
19/71	PR63LLO1 / 62/3	0,57
19/72	PR63LLO1 / Зоря	0,19
19/73	Слобож / Polar CL	0,34
19/74	Слобож / L25	0,15
19/75	UE*1021 / L25	0,59
19/76	Стадіон / PR63LLO1	0,53
19/77	X51Б / 64/3	0,28
19/78	X51Б / 56/3	0,28
19/79	X51Б / S2K670	0,33
19/80	X51Б / Чк.гігант	0,36

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

Усього 73 (сімдесят три) міжсортові гібриди. Кількість насіння одного зразка – 50 штук.

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