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GERMINATION OF PUMPKIN SEEDS UNDER CD STRESS

Chen Rui
PhD student
Sumy National Agrarian University, Sumy, Ukraine
ORCID: 0009-0007-3414-241X
cr15225939975@163.com

Dubovyk Volodymyr
PhD (Agricultural Sciences)
Sumy National Agrarian University, Sumy, Ukraine
ORCID: 0000-0002-2880-7047
dvi_docent@ukr.net

Cadmium is a non essential element for plant growth, and its accumulation in plants can cause damage to a certain extent. The accumulation of heavy metals not only inhibits the growth and development of plants, but also threatens human health through the food chain. Therefore, the problem of heavy metal pollution cannot be ignored. Studies have shown that grafting can reduce the accumulation of cadmium in Pumpkin fruits, such as eggplants, providing new ideas for mitigating the impact of heavy metal pollution on agricultural products. Pumpkin (Cucurbita moschata (Duchesne ex Lam.) Duchesne ex Poir.) as an important vegetable crop, it has a well-developed root system and good affinity, and is often used as a grafting rootstock for melon crops. Seed germination is a crucial stage for evaluating plant cadmium tolerance. The evaluation of plant tolerance to heavy metals during germination is a widely recognized method internationally.

Therefore, this study investigated the effects of cadmium stress on pumpkin seed germination and the tolerance of different pumpkin varieties to heavy metal cadmium under the same concentration (8mg/L^{-1} Cd^{2+}) of heavy metal cadmium stress, in order to screen pumpkin resources that are tolerant to heavy metal cadmium stress and provide a basis for further screening of pumpkin rootstocks that are tolerant to heavy metal cadmium pollution.

The research results indicate that cadmium has a significant impact on the germination status of some combinations of seeds, while the impact on other combinations is relatively small, such as: Hetou a2×041-1, Hetou a2×360-3, and Yanbian-2×041-1 have high relative germination rates, with Yanbian-3×041-1 having the highest relative germination potential, Yanbian-3×041-1 having the most prominent relative germination index, and Yanbian-3×Lingchuan c1 having the highest relative vitality index. Overall, 360-3×041-1 and Hetou a2×360-3 are at a relatively high level in terms of relative germination rate, relative germination index, and relative vitality index, while Yanbian-2×041-1 and Yanbian-3×041 are at a relatively high level. The three indicators of relative germination potential, relative germination index, and relative vitality index are all at a relatively high level, indicating that they are relatively less affected by cadmium. According to the analysis of membership functions, the average value of the membership function for combinations 360-3×041-1 is the highest, followed by Yanbian-3×041-1, Hetou a2×360-3, Yanbian-2×041-1, Yanbian-4×Lingchuan c1, Yanbian-3×Lingchuan c1, and Hetou a2×041-1. The average value of the membership function is above 0.70, indicating good growth status and good Cd tolerance. Therefore, seven pumpkin hybrid combinations with strong Cd tolerance were finally selected.

Key words: *Cadmium stress, pumpkin, hybrid combination, seed, germination rate, relative germination potential, relative germination index, relative vitality index, membership function, cadmium tolerance.*

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Introduction. In recent years, the unreasonable use of chemical pesticides and fertilizers has led to the continuous deterioration of the agricultural ecological environment (Li Liupeng et al., 2014). In agricultural production continuous cropping, excessive fertilization, the extensive use of pesticides, and non-standard application of farmyard manure have all contributed to a series of obstacles, such as soil secondary salinization and heavy metal pollution (Han Lili et al., 2017; Peng Qingtang et al., 2018; Zheng Wenjuan et al., 2019). Among these pollutants, heavy metal (cadmium, in particular) stands out due to its high mobility, high toxicity, and the vast area it contaminates (Chen Bihua et al., 2017). It is recognized as one of the most serious heavy metal contaminants in pollution sources (Gan Tingting et al., 2021; Zhang Yangyang et al., 2021).

There have been many reports on the physiological toxicity of cadmium stress on plants, mainly focusing on crops such as wheat (Meng Guiyuan et al., 2016), corn (Sun Yali et al., 2017), foxtail millet (Zhang Panpan et al., 2020), chili pepper (Long Chunli et al., 2021), and ryegrass (Li Meifang et al., 2021).

Cadmium is a non-essential element for plant growth, and its accumulation can cause damage to a certain extent by inhibiting seed enzyme activity, cadmium reduces germination rate and even causes plant death (Tian Xiaoxuan, 2020; Tang Guangmei et al., 2021; Chen Li, 2017). The accumulation of heavy metals not only inhibits the growth and development of plants, but also enters the human body through the food chain, thereby threatening human health. Its harm is serious and complex, and therefore, the problem of heavy metal pollution cannot be ignored (Haider et al., 2020).

The remediation and safe utilization of cadmium-contaminated soil have attracted high attention in the industry. The use of hyper accumulating plants for cadmium pollution remediation is a common technology for treating heavy metal-contaminated soil. This technology is characterized by its simplicity, low cost, and significant economic and ecological benefits, and has been widely recognized and applied (Li Mengying et al., 2024). Research reports have identified several Cd hyperaccumulating plants, including *Viola baoshangensis* (Liu Wei et al., 2003), *Brassica juncea* (Liu Shasha et al., 2018), *Solanum nigrum* (Lei Long et al., 2021), *Tlaspi caerulescens* (Han Lu et al., 2007), *Sedum alfredii Hance*, and *Phytolacca acinosa* (Long Yumei et al., 2019). However, these species have limitations such as relatively small biomass or narrow regional adaptability. Therefore, it is necessary to screen and cultivate plants which are tolerant to cadmium, with high ability to this element accumulation, large biomass potential, and high adaptability, available for cadmium-contaminated soil remediation.

Pumpkin (*Cucurbita moschata*) is popular crop for its high nutritional value and taste. As an important vegetable, pumpkin has a well-developed root system and good affinity, often serving as a grafting rootstock for melon crops. Currently, facility vegetables mainly overcome continuous cropping obstacles through grafting. Studies have found that grafting can reduce the accumulation of cadmium in eggplant fruits (Lou Wei, 2010), suggesting its potential use in cadmium-contaminated soil remediation.

Seed germination serves as a critical stage for evaluating plant tolerance to cadmium, and internationally, assessing plant tolerance to heavy metals during this stage is a widely recognized method (Zhang Panpan et al., 2020). Currently, there are relatively few research reports on pumpkin resources that exhibit strong cadmium tolerance.

Therefore, the aim of study to investigate the effect of cadmium stress on pumpkin seed germination and evaluated the tolerance of various pumpkin varieties to the heavy metal under the same concentration of cadmium. The objective was to screen pumpkin resources that exhibit tolerance to cadmium stress, thereby providing a basis for further selection of pumpkin rootstocks that are resistant to heavy metal cadmium pollution.

Materials and methods. 19 pumpkin hybrid combinations were tested, all provided by the Pumpkin Research Group of the College of Horticulture and Landscape Architecture, Henan University of Science and Technology (Table 1).

The experiment was conducted from 5th to 15th of October, 2022, in the experimental building at the College of Horticulture and Landscape Architecture, Henan University of Science and Technology.

Well-filled symmetrical pumpkin seeds were selected, firstly they were washed with tap water, and then were soaked in distilled water for three hours. Seeds were placed on the filter paper in a 90-mm diameter culture dish and distributed evenly with 15 seeds per dish. Then an appropriate amount of 8 mg/L Cd solution to the experimental group of culture dishes was added, while to the control group culture dishes only distilled water was put. Each treatment will be repeated three times. Culture dishes were placed in an artificial incubator for germination at a temperature of 28 ± 1 ℃, with the emergence of an embryonic root of 0.2 cm in length serving as the germination criterion. Continuously the number of germinated seeds were counted from 2 to 5 days, and then the embryonic root length was measured and recorded daily.

Indicators in experiment.

(1) Germination rate=number of seeds germinated within 5 days/total number of seeds×100%

(2) Germination potential=number of seeds germinated within 3 days/total number of seeds×100%

(3) Germination index=∑ Nt/Tt

Table 1

Resources of different pumpkin hybrid combinations

Hybrid combinations	100 grain weight, g	Seed color	The number of seeds per pumpkin
360-3×041-1	12.648	white	290
360-3×Lingchuanc1	11.35	white	247
Fangshan×Lingchuanc1	6.739	yellow and white	59
Hetoua2×041	8.75	yellow and white	352
Hetoua2×360-3	9.69	yellow and white	303
Hetoua2×Lingchuanc1	10.08	yellow and white	282
Renhe-1×360-3	7.942	white	295
Renhe-1×Lingchuanc1	9.84	yellow and white	108
Renhe-2×041	13.25	white	273
Renhe-2×360-3	7.086	yellow and white	170
Renhe-2×Lingchuanc1	13.03	yellow and white	359
Yanbian-2×041	9.57	white	135
Yanbian-2×360	10.585	pale	145
Yanbian-3×041-1	9.91	pale	248
Yanbian-3×360-3	12.296	pale	246
Yanbian-3×Lingchuanc1	10.31	yellow and white	304
Yanbian-4×041	10.899	white	325
Yanbian-4×360-3	8.113	pale	460
Yanbian-4×Lingchuanc1	8.85	yellow and white	434

In the formula: N_t : germination number; T_t : germination time (d).

(4) Vitality index=R×∑ Nt/Tt

In the formula, R : represents the length of the embryonic root of the sprout (cm).

(Relative values of all indicators=Cd treated value/ control value x 100%)

(5) Comprehensive evaluation of Cd tolerance by membership functions

Use membership function values to comprehensively evaluate the Cd tolerance of various measured indicators. If the measured indicators are positively correlated with the Cd tolerance of pumpkin seedlings, use the formula *R (Xi)=(Xi Xmin)/(Xmax Xmin)* to calculate; On the contrary, calculate using the formula *R (Xi)=1-(Xi Xmin)/(Xmax Xmin)*. The larger the average membership function value obtained, the better the growth status and the stronger the cadmium resistance.

In the formula, Xi represents various indicators of pumpkin seedlings, Xmin represents the minimum value of indicators, and Xmax represents the maximum value of indicators

For data processing Excel 2003 (data summaring), DPS 7.55 (dana analysis) were applied, one-way ANOVA and Duncan test were used to compare significant differences (P<0.05), and Excel 2021 – for making statistical charts.

Results. *The effect of cadmium stress on the germination status of pumpkin seeds in different hybrid combinations.* Under cadmium stress, different pumpkin hybrid combination seeds exhibited significant differences in tolerance. The germination status of some seed combinations was significantly affected by cadmium, while others were relatively less impacted. According to Table 2, Hetoua2×041-1, Hetoua2×360-3, and Yanbian-2×041-1 had higher relative germination rates among 19 pumpkin hybrid combinations. Next Yanbian-4×Lingchuanc1, Yanbian-3×Lingchuanc1, 360-3×Lingchuanc1, and 360-3×041-1 were. However, the relative germination rate of Yanbian-4×360-3 was the lowest, at only 42.73%.

Regarding the relative germination potential, Yanbian-3×041-1 ranked first, followed by Yanbian-2×041-1, Hetoua2×360-3, 360-3×041-1, Yanbian-3×360-3, Yanbian-3× Lingchuanc1, and Yanbian-4×Lingchuanc1. The relative germination potential of Yanbian-4×360-3 was the lowest, at only 51.46%.

The relative germination index of Yanbian-3×041-1 was the most prominent, followed by Yanbian-4×Lingchuanc1 and 360-3×041-1. Among them, the relative germination index of Hetoua 2×Lingchuanc1 was the lowest, indicating that it was most affected by cadmium stress.

Lastly, the relative vitality index of Yanbian-3× Lingchuanc1 was the highest, followed by 360-3×041-1, Hetoua 2×041-1, and Hetoua 2×360-3. The relative vitality index of Yanbian-4×360-3 was the lowest, at 52.12%.

The membership function values of pumpkin seed germination in different hybrid combinations under cadmium stress.

The level of Cd tolerance of seeds cannot be evaluated solely based on a single indicator. It is necessary to comprehensively evaluate the level of Cd tolerance through the membership function values of seed germination rate, germination vigor, germination index, and vitality index. Analyzes the germination status of 19 pumpkin hybrid combinations using membership functions were presented (Table 3). The average membership function

Table 2

Seed germination status of different pumpkin hybrid combinations under cadmium stress

Hybrid combinations	Relative germination rate, /%	Relative germination potential, /%	Relative germination index, /%	Relative Vitality Index, /%
360-3×041-1	63.41±5.43a	69.09±1.1bc	73.86±3.84ab	81.99±4.72a
360-3×Lingchuanc1	63.6±3.92ab	65.07±0.75def	67.06±0.53bcde	73.43±5.97abc
Fangshan×Lingchuanc1	54.73±1.17abc	56.94±3.26h	60.83±6.12de	62.61±6.72cde
Hetoua2×041	66.81±0.12abc	64.22±1.55efg	67.32±3.44abcde	78.99±1.74ab
Hetoua2×360-3	68.19±4.05abc	70.24±0.76b	69.85±1.14abcd	76.9±2.17ab
Hetoua2×Lingchuanc1	59.07±2.66abc	57.44±0.63h	59.68±6.16e	66.45±7.13bcd
Renhe-1×360-3	61.12±7.75abc	57.79±3.18h	61.52±7.3de	71.98±6.17abc
Renhe-1×Lingchuanc1	46.33±1.28bcd	58.06±2.95h	63.07±3.26cde	56.06±6.38de
Renhe-2×041	59.08±2.26cde	61.65±0.49g	62.37±6.35cde	68.29±7.29bcd
Renhe-2×360-3	54.07±1.42cde	57.74±1.47h	60.14±3.3e	62.81±6.23cde
Renhe-2×Lingchuanc1	54.87±2.26cde	63.61±1.11fg	62.72±1.63cde	61.72±7.02cde
Yanbian-2×041	65.53±3.52def	73.96±2.13a	71.76±2.56abc	71.5±1.51abc
Yanbian-2×360	54.84±1.2def	55.64±2.77h	61.61±3.57de	62.86±7.01cde
Yanbian-3×041-1	59.46±7.54def	75.12±0.42a	76.46±12.87a	70.89±16.58abc
Yanbian-3×360-3	51.58±1.85ef	67.7±2.34bcd	63.89±2.15cde	62.64±6.91cde
Yanbian-3×Lingchuanc1	64.43±2.36fg	67.4±0.67bcde	67.95±1.63abcde	83.58±6.47a
Yanbian-4×041	50.44±5.06fg	61.23±0.42g	61.71±3.69de	57.31±5.79de
Yanbian-4×360-3	42.73±1.56gh	51.46±1.39i	59.56±5.06e	52.12±5.77e
Yanbian-4×Lingchuanc1	64.92±1.46h	66.31±1.6cdef	76.18±2.89ab	73.15±7.65abc

Note: The different letters within the same index indicate significant differences between the rootstocks (P<0.05).

of 360-3×041-1 hybrid was found to be the highest, followed by Yanbian-3×041-1, Hetoua 2×360-3, Yanbian-2×041-1, Yanbian-4×Lingchuanc1, Yanbian-3×Lingchuanc1, and Hetoua2×041-1. The average membership function values of these top seven hybrid combinations are all above 0.70. In contrast, the average values of the membership functions for Renhe-1×Lingchuanc1 and Yanbian-4×360-3 are relatively low, both below 0.20. Overall, the hybrid combinations mentioned above, except for Renhe-1×Lingchuanc1 and Yanbian-4×360-3, exhibited satisfactory Cd resistance.

Discussion. Seed germination is a critical stage in the plant life cycle. Cd at different concentrations exerts varying effects on plant seed germination, generally showing a pattern of 'low promotion and high inhibition', that is, with the increase of cadmium ion concentration, germination rate, germination potential, and germination index exhibit a trend of first increasing and then decreasing. However, not all plant seeds respond the same way to different concentrations of Cd^{2+} (Dong Lili, 2023).

In some plants, low concentrations of Cd promote seed germination, while high concentrations inhibit this indicator. For example, the germination of lemon seeds was promoted by Cd at low concentrations (10 mg/l^{-1} of Cd^{2+}), but was inhibited at higher concentrations (100 mg/l^{-1} of Cd^{2+}) (Yan et al., 2019). *Brassica napus* was not significantly affected by low concentrations of Cd^{2+}. However, when the concentration of Cd^{2+}exceeds a certain critical value, it will inhibit plant growth (Liu et al., 2016). Cadmum concentraion of 1–8 mg/l^{-1} has no significant inhibitory effect on the germination of tobacco *(Nicotiana tabacum*) seeds, while concentrations of 16 mg/l^{-1} of Cd^{2+}or higher significantly inhibit their germination (Melis, 2009). It has been validated on species such as alfalfa (Bao Qiongli et al., 2020), bok choy (Wang Dihua et al., 2021), purslane (Zhu Hongxia et al., 2020), *Asteraceae* species (Wu Rangxiao et al., 2020), and devil's needle grass (Yang Yun et al., 2022).

Through seed germination experiments with 19 pumpkin hybrid combinations, it was found that 360-3×041-1 and Hetoua 2×360-3 were at a relatively high level in terms of germination rate, germination index, and vitality index, while Yanbian-2×041-1 and Yanbian-3×041-1 were at a high level in terms of relative germination potential, relative germination index, and relative vitality index, indicating that they were relatively less affected by cadmium. According to the membership function analysis of seed germination status, the average membership function of 360-3×041-1 is the highest, followed by Yanbian-3×041-1, Hetoua2×360-3, Yanbian-2×041-1, Yanbian-4×Lingchuanc1, Yanbian-3×Lingchuanc1, and Hetoua2×041-1. The average membership function of the top seven hybrid combinations has reached 0.70 or above, this indicated that the plant was in good growth condition and had good Cd tolerance.

Conclusions. Through seed germination experiments on 19 different pumpkin hybrid combinations, it was found that cadmium had a significant impact on the germination status of some combinations, while the impact on other combinations was relatively small. For example, 360-3×041-1 and Hetou a2×360-3 had high levels of relative germination rate, relative germination index, and relative vitality index, while Yanbian-2×041-1 and Yanbian-3×041-1 had high levels of relative germination potential, relative germination index, and relative vitality index, indicating that they were relatively less affected by cadmium. According to the membership function analysis of seed germination status, the average membership function of 360-3×041-1 is the highest, followed by Yanbian-3×041-1, Hetou a2×360-3, Yanbian-2×041-1, Yanbian-4×Lingchuan c1, Yanbian-3×Lingchuan c1,

Table 3

Values of membership function of seed germination of different pumpkin hybrid combinations under cadmium stress

Hybrid combinations	Germination percentage	Sprouting potential	Germination index	Vitality index	Average value	Sort
360-3×041-1	0.81	0.75	0.85	0.95	0.84	1
360-3×Lingchuanc1	0.82	0.58	0.44	0.68	0.63	8
Fangshan×Lingchuanc1	0.47	0.23	0.08	0.33	0.28	15
Hetoua2×041	0.95	0.54	0.46	0.85	0.70	7
Hetoua2×360-3	1.00	0.79	0.61	0.79	0.80	3
Hetoua2×Lingchuanc1	0.64	0.25	0.01	0.46	0.34	13
Renhe-1×360-3	0.72	0.27	0.12	0.63	0.43	10
Renhe-1×Lingchuanc1	0.14	0.28	0.21	0.13	0.19	18
Renhe-2×041	0.64	0.43	0.17	0.51	0.44	9
Renhe-2×360-3	0.45	0.27	0.03	0.34	0.27	16
Renhe-2×Lingchuanc1	0.48	0.51	0.19	0.31	0.37	12
Yanbian-2×041	0.90	0.95	0.72	0.62	0.80	4
Yanbian-2×360	0.48	0.18	0.12	0.34	0.28	14
Yanbian-3×041-1	0.66	1.00	1.00	0.60	0.81	2
Yanbian-3×360-3	0.35	0.69	0.26	0.33	0.41	11
Yanbian-3×Lingchuanc1	0.85	0.67	0.50	1.00	0.76	6
Yanbian-4×041	0.30	0.41	0.13	0.16	0.25	17
Yanbian-4×360-3	0.00	0.00	0.00	0.00	0.00	19
Yanbian-4×Lingchuanc1	0.87	0.63	0.98	0.67	0.79	5

and Hetou a2×041-1. The average membership function of the top seven hybrid combinations has reached 0.70 or above, this indicated that the plant was in good growth condition and had good Cd tolerance. Therefore, seven ones with high cadmium tolerance were ultimately selected, namely 360-3×041-1, Yanbian-4×Lingchuan c1, Yanbian-3×360-3, Yanbian-2×041-1, Yanbian-3×Lingchuan c1, Hetou a2×041-1, and Hetou a2×360-3.

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Чен Жуй, *аспірант, Сумський національний аграрний університет, м. Суми, Україна*

Дубовик В. І., *кандидат сільськогосподарських наук, доцент, Сумський національний аграрний університет, м. Суми, Україна*

Схожість насіння гарбуза в умовах Cd стресу

Кадмій є не обов'язковим елементом для росту рослин, і накопичуючись в організмі до певної міри, може завдати шкоди рослинам. Накопичення важких металів не тільки пригнічує ріст і розвиток рослин, а й загрожує здоров'ю людини через харчовий ланцюг, тому проблему забруднення важкими металами не можна ігнорувати. Дослідження показали, що накопичення кадмію в баклажанах та інших Плоди гарбуза можна зменшити за допомогою щеплення, що дає нову ідею для пом'якшення впливу забруднення важкими металами на сільськогосподарську продукцію. Гарбуз (Cucurbita moschata (Duchesne ex Lam.) є важливою овочевою культурою з добре розвиненою кореневою системою і хорошою спорідненістю, і часто використовується як щеплений підщепу для баштанних культур.

Таким чином, було вивчено вплив різних сортів гарбуза на схожість насіння та толерантність до кадмію за однакової концентрації стресу кадмію (8 мгL^{-1}Cd^{2+}), а також проведено скринінг ресурсів гарбузів, стійких до кадмію, що забезпечило основу для подальшого скринінгу коренеплодів гарбуза, стійких до кадмію.

Результати показали, що кадмій суттєво вплинув на статус схожості насіння деяких комбінацій, тоді як інші комбінації зазнали відносного впливу. Хету а2×041-1, Хету а2×360-3 та Янбянь-2×041-1 мали високі відносні показники схожості, Янбян-3×041-1 посів перше місце, Янбян-3×041-1 відносний індекс схожості є найбільш помітним, а Янбян-3× Лінгчуань с1 мав найвищий відносний індекс схожості. Індекс відносної бадьорості трьох індексів був на високому рівні, а три індекси Янбянь-2×041-1 та Янбян-3×041-1 були на високому рівні за відносним потенціалом проростання, відносним індексом схожості та індексом відносної бадьорості, що вказує на те, що вони були відносно менш схильні до впливу кадмію. Згідно з результатами аналізу функцій приналежності, середнє значення функції приналежності 360-3×041-1 було найвищим, за ним йшли Яньбянь-3×041-1, Хету а2×360-3, Яньбянь-2×041-1, Яньбянь-4×Лінчуань с1, Яньбянь-3×Лінгчуань с1, Хету а2×041-1. Середнє значення функції членства вище 0,70, що вказує на хороший статус росту і хорошу переносимість Cd. Тому нарешті було відібрано сім гібридних комбінацій гарбуза з сильною толерантністю до Cd.

Ключові слова: *стрес кадмію, гарбуз, гібридна комбінація, насіння, швидкість проростання, відносний потенціал проростання, відносний індекс схожості, індекс відносної сили, функція членства, толерантність до кадмію.*