Genetic Toxicity of Solidago canadensis

Zhongling Gong,Wenjie Xu

Gas group co., LTD of Shenzhen City · 518049 · china

Abstract: Solidago decurrens is a plant of Asteraceae, which is mainly produced in southern China. It is widely used cut flower material. It is known as the malignant weed Solidago canadensis (Solidago canadensis) with the genus Flora, so its application of ecological security has been a big debate. In this paper, the allelopathic effect of the water extract of Solidago macrophylla was studied by using the micronucleus technique of Vicia faba root tip as the experimental material. The aim of this study was to analyze the genetic toxicity of Solidago canadensis. The results showed that: (1) The water extract of Solidago canadensis could decrease the mitotic index and interfere with the normal process of mitosis, where the decreased rate was positively correlated with the concentration of water extract and treatment time. (2) The effects of water extract on the root tip of Vicia faba were significant increases with time and concentration effect (3) Achilla glauca extract could make the chromosome bridge, chromosome fragment, chromosome lag in the root tip cells of Vicia faba and increase in micronucleus rate. The results showed that the water extract of Solidago canadensis had different degree of inhibition and damage to silkworm root tip cells; it also had some genetic toxicity and rapid diffusion ability.

Key words: Solidago canadensis; Genotoxicity; Vicia faba root tip; micronucleus

1 Introduction

Ailaceae (Asteraceae) is a perennial herb, mainly grown in China's east, south, southwest, Shaanxi, Taiwan and other places. The seeds of these yellow flowers are very small, they were long hairy, dandelion-like seeds as the wind drift,it can spreads widely, upon reaching the soil it will initiate the grow of new plants. The old plant withered soon after the seeds are formed and there will be dozens of young branches grow around its roots. Seedling recurs on the next flowering season.

In recent years, domestic and foreign reports on the research of Huanghua mainly focus on the chemical composition of the yellow flower [1-2], physical and chemical analysis [3], medicinal ingredients and pharmacological effects [4], and allelopathy [5-6]. Research methods are also different.

Natural plants are rich in terpenoids, it had been confirmed that the plant derived terpene compounds will produce allelopathy and causes some effect on other plants. It is known that the essential oil of Solidago canadensis is rich in monoterpenes, sesquiterpenes, monoterpene oxides, sesquiterpenes and other components [7].

There are many reasons for the successful invasion of alien plants, in which allelopathy plays a very important role in the process of plant invasion [8], allelochemicals can significantly affect plant cell growth and differentiation [9]. At present, there are a lot of bioassay methods used in the study, which causes the lack of standards and even the same laboratory research reports using bio-determination methods are not the same hence, different allelopathy between the results difficult to compare. In recent years, there are more domestic allelopathy researches, where the researchers need more understanding of domestic and foreign methods of bioassay and the adaptability of these methods. Choosing the appropriate biometric method is one of the keys in the study of allelopathy [10]. Vicia faba has been used since the establishment of Degressi in 1982. It is widely used for the mutagenicity of chemical toxic substances and environmental pollutants in the environment of water, soil and atmosphere. As early as 1986, the State Environmental Protection Administration already included Vicia faba root tip micronucleus test technology in the 'Environmental Monitoring Technical Specifications' for water environmental monitoring technology.

At present, several indicators of genetic toxicity research are mitotic index, micronucleus rate, chromosome aberration such as Chen Liping [11], Du Feng moved [12], Zhou Xiaokui [13] and Fan Xuetao [14] The genetic toxicity of alien invasive plants was determined by using the indexes above. Gao Beiye, Xie Huanxun, Zhou Mingming [15] had been through the broad bean micronucleus technology to evaluate the safety of the Dan and provide the basis for its comprehensive utilization. The mutagenic effect of Juglans mandshurica extract extract (JMME) was studied by using micronucleus test (MNT) method in mouse bone marrow cells, and the effects of Juglans mandshurica extract extract (JMME). It was also used to evaluate whether Juglans mandshurica is genotoxic.

Solidago canadensis L. is a widely used cut flower material, because it is known as the malignant weeds of Solidago canadensis L. (Solidago canadensis L.) with the genus Flora, so its application of ecological security has been a big debate. In order to scientifically and rationally use Solidago canadensis, it is necessary to study the genotoxicity of the plant extensively.

2. Research methods

2.1 Experimental materials

Marketed broad bean seeds, commercially available yellow flower. Distilled water, Carnos fixed solution (anhydrous ethanol: glacial acetic acid = 3: 1), modified phenol magenta solution, 70% ethanol, 1 mol / L hydrochloric acid, 0.5% KMnO4 solution.

2.2 Experimental equipment

Microscope, slides, coverslips, filter paper, paper tweezers, anatomical needles, incubators, scissors, graduated cylinders, white disks, gauze, water baths, ovens, label paper

2.3 Experimental steps

2.3.1 Preparation of Solid Extract of Solidago canadensis

Take the healthy and fresh leaves, first rinse with water then dry. Next, use scissors to cut it, take its weight and put into the conical flask, according to 1g weight per 4mL of the water. Then, carry out an intermittent oscillation (23oC) extraction, extract the solution after 48 hours by using three layers of clean gauze to extract the solution (concentration of 0.25 g / mL). Then, the extracted solution were diluted with distilled water to 0.025 g / mL, 0.050 g / mL, 0.075 g / mL, and 0.100 g / mL and kept in the refrigerator for use [11-16].

2.3.2 Selection of beans, disinfection and soaking germination

According to the State Environmental Protection Administration 'water and wastewater monitoring and analysis methods' in the 'Vicia faba root tip micronucleus test' method and did some improvement.

Select the full, uniform size, non-damaged broad bean seeds. Wash with tap water before disinfect it with 0.5% KMnO4 for 30 min then, wash with distilled water for 3 to 5 times. Soak the beans in beaker containing distilled water for 24 hours at 23 ° C and change the water for at least twice (the water should be warmed at 23 ° C). After the expansion of seed, loosely wrap it with gauze around the white disk to maintain humidity, then put into a 23oC incubator for germination of 12-24 hours (no light culture), until the root grow up to 2-3mm. Then take those good buds seeds and put it onto the white disk covered with filter paper. Continue the 23oC for germination for about 36-48 hours [17]. Choose the root length of the primary root which is about 1-2cm long, beans with well-developed root hair were randomly divided into five groups, where each porcelain dish has 40-50 tablets.

2.3.3 Processing

The apices were treated with 40 mL of a yellow flower extract of 0.025 g / mL, 0.05 g / mL, 0.075 g / mL and 0.1 g / mL respectively. At the same time, another petri dish was treated with distilled water as a control. The rats were treated with 10-15 capsules for 24 h, 48 h, 72 h [11-16] (cultured at 23 ° C, 80% humidity, 14 h / d for 3 days).

2.3.4 Root tip recovery culture

A total of 10 broad bean seeds were extracted from each treatment at 24 h, washed three times with distilled water for 3 min each, and then transferred to a 23 ° C incubator for 24 h.

2.3.5 Fixed cell apical

The recovered seeds were cut from the tip of the root tip by a 1-1.5 cm long root into the jar and fixed with Carnot's fixative for 2-24 hours and stored in 70% ethanol solution at 4 ° C by saving in the refrigerator. Fixation was done in order to kill the cells quickly and to make the nucleus, organelles in the normal structure as they were alive, real state, so that cells are permanently in the split cycle of a certain period. The traditional practice is put into the Carnos liquid, the liquid used for cytology production, especially in fixing chromosomes, centrosomes, DNA and RNA, fixed for a variety of dyeing. In this experiment, the fixative solution can prevent the hardening and shrinkage of ethanol and increase the penetration force.

2.3.6 Producer

Wash: Wash the young roots with distilled water for 2-3min. Washing the residue at the apical Karno's fixation.

Acid solution: Add 1mol / L hydrochloric acid immersed young roots 60oC water bath 10min, so that the root softening. Dissociation is the separation of the pectin layer between the cells so that the cells are separated from each other, the cells are easily dispersed and the original shape of the cells is maintained. In the experiment, we observed that it is important to grasp the dissociation time accurately. First of all, it must be completely dissociated. Otherwise, it is easy to form the tissue block regardless of the dyeing or the production effect. But the dissociation time should not be too long; otherwise the cell structure will be destroyed and hence, cannot observe the split. Dissociation criteria based on material soft cells easy to disperse prevail, the appropriate dissociation time is 3 to 5 minutes. It should also be based on the concentration of solution used and the ambient temperature.

Wash: Wash away with distilled water before and after dissociation. Experiments require dissolution of the root tip after dissociation. The purpose is to wash away the acid and to avoid neutralization reaction with the alkaline dye which is conducive in chromosome coloring.

Dyeing and tableting: Cut 1 to 2mm long root tip and place it on the slide, cut it into two points longitudinally using a blade, drop a drop of dye, and dye for 5 to 8min with coverslips and tablet. Dyeing is to shade the color of cytoplasm and chromosomes differently so, the chromosome behavior can be clearly observed. Press the cover is covered with coverslips, with a filter paper pressure on the cover of the two corners. On flat surface, gently tap the cover material with a rubber pen from the middle to the surrounding so, the material can discrete Into a uniform fine mist, to avoid the material crushed or cause uneven cell accumulation which can cause impact in observation.

2.3.7 Mirror and counting

After the conventional production, the slide specimen was observed under the microscope at low magnification, and look for the parts where the tissue were well dispersed, large nucleus with the split phase for further the observation under high magnification. Then, according to the identification criteria starts counting and taking pictures. If the chromosomal dispersion is uneven and difficult to distinguish and count, you can remove the film, flat it on a flat surface, with your fingers across the absorbent paper gently press on the coverslip. If operated carefully with moderate force, you can easily get a good specimen for observation, counting and photography.

The allelopathic effect index (RI) is calculated by reference to Williamson [18] and so on: RI = 1-C / T, where C is the control value, T is the treatment value and RI is the allelopathic effect (RI> 0 for the promotion, RI <0 for the inhibition, the absolute value of the size of the same intensity).

2.3.8 Statistical processing

Duncan's test was performed on the data obtained.

2.4 operating points

2.4.1 Preparation of reagents

For the preparation of toxic reagents should wear disposable plastic gloves, will be equipped with a good solution stored in the refrigerator at 4 ° C, after preparation to wash their hands.

2.4.2 Germination treatment

Vicia seed germination needs warm and humid condition and should pay attention to moisturizing insulation. However, cannot completely immerse the seeds, otherwise it will affect its breathing hence, when the grass paper dry, should add a small amount of water to grass paper water.

2.4.3 Induction

Fully immersed the root of the plant in the solution will achieve better results. In addition, the processing time should be accurate. If too long it will increase the root tip micronucleus rate which can results in error.

2.4.4 To restore culture

After vicia faba root tip cells treated with mutagens, DNA damage occurs. However, if some cells do not split or if it still maintains the original single-cell nuclear state then there is no micronucleus formed. Only by the resumption of culture and mitosis, the DNA damage can be expressed whereby this performance is the formation of micronuclei. So, it must be restore by culture.

The processed beans must be washed thoroughly to avoid any remaining yellow flower extract on it, so it is best to wrap it with gauze and rinse with distilled water. Then, place it into the 23oC incubator to make the effect more obvious.

3. The experimental results

3.1 Effects of Extracts from Solidago canadensis on Mitosis of Vicia faba Root Tip Cells

The effect of Solidago canadensis on the mitotic index of Vicia faba root tip cells is shown in Table 1, Fig.

Table 1. Effects of Araceae Extract on Mitosis of Vicia faba Root Tip

Concentration of aqueous extracts（g/mL）	24h	48h	72h

mitotic index
(%) RI mitotic index
(%) RI mitotic index
(%) RI
0.000
0.025
0.050
0.075
0.100 9.46
6.20**
6.04**
5.44**
2.56** —
-0.526
-0.566
-0.739
-2.695 7.18
6.32**
6.08**
4.58**
2.36** —
-0.136
-0.181
-0.568
-2.042 4.52
5.64**
5.54**
2.80**
2.02** —
0.199
0.184
-0.614
-1.238

Note: * and ** represent the difference between the 0.05 and 0.01 levels of one-dimensional analysis of variance

Figure 1. Effect of Araceae Extract on Mitosis of Vicia faba Root Tip

From Table 1 and Figure 1, it was found that the extract of Solidago canadensis had a significant inhibitory effect on the mitotic index of Vicia faba root tip cells. The inhibitory effect of extract on mitosis was positively correlated with the treatment time and the concentration of extract, where the increase and the role of time to extend the suppression effect (P <0.01). After processing (72 h) of 0.025 g / mL and 0.050 g / mL, the mitotic index slightly higher than that of the control group (P <0.01), this may be due to the presence of a certain amount of allelochemicals. The leachate of the leachate is small, to a certain extent, it is able to slow down the water stress, but with the increase in the concentration of allelochemicals it causes this mitigation effect.

The RI value of the mitotic index of the water extract of Solidago monocytogenes also showed a significant inhibitory effect on the mitotic index of Vicia faba root tip cells.

3.2 Effects of Extracts from Solidago canadensis on Micronucleus Rate in Vicia faba Root Tip Cells

Table 2. Effects of Araceae Extract on Micronucleus Rate in Vicia faba Root Tips

concentration
of aqueous extracts（g/mL） 24h 48h 72h
The frequency
of micronucleus
(%) RI The frequency of micronucleus
(%) RI The frequency of micronucleus
(%) RI
0.000
0.025
0.050
0.075
0.100 1.0
3.6**
4.6**
4.8**
5.6** —
0.722
0.783
0.792
0.821 2.6
4.8**
6.2**
10.0**
10.4** —
0.458
0.581
0.740
0.750 5.2
6.8*
9.4**
10.6**
10.8** —
0.235
0.447
0.509
0.519

Note: * and ** represent the difference between the 0.05 and 0.01 levels of one-dimensional analysis of variance

Fig.2 Effect of extract of Solidago canadensis on micronucleus rate of root tip of Vicia faba

Achromatic water extract induces a higher frequency of micronuclei (Table 2, Figure 2), also has a double dependency on the treatment time and the concentration of the extract. With the increase of treatment time and the increase of concentration, the micronucleus ratio of each concentration was significantly increased. Compared with the control group, the micronucleus ratio of the root tip of the treatment group reached the extremely significant level (P <0.01).

3.3 Effects of Solid Extract of Solidago canadensis on Chromosomal Abnormality of Vicia faba Root Tip Cells

There were many abnormalities in the chromosomes of the root tips of the broad bean, such as micronucleus, chromosome bridge, chromosome fragment, chromosome adhesion and chromosome lag. There are micronuclei and micronuclei in the various stages of cell division (Figure 3), the number is different.

???????????? ?????

?Single micronucleus Double micronucleus Pre

– micronuclei

(interphase micronucleus)

??

Mid - term micronucleus Late micronucleus Late chromosome single bridge

?

Late chromosome double bridge Late chromosome fragment Late chromosome adhesion

Late chromosome retention Late - stage micronucleus Late chromosome

Figure 3. Chromosome aberration of Vicia faba root tips induced by aqueous extract of Solidago canadensis

4. Discussion

The mitotic index represents the ratio of the number of apical dividing cells to the total number of cells in the stratified area. The higher the value, the higher the root tip cell division is, the better the growth. Allelochemicals can inhibit the formation of spindle filaments in the process of mitosis, destroying the normal structure and function of the spindle filaments, thereby inhibiting cell division [19]. The results of this study show that the mitotic index of Vicia faba root tip cells is decreased under the action of Araceae water extract, which may be the process of infestation. The vegetative organs secrete allelopathic substances into the soil and inhibit the growth of other plant roots around them leading to other plant growth and development, or even death.

The results showed that the water extract of Solidago canadensis could induce the higher frequency of micronuclei. The micronucleus ratio of the water extract treatment group was significantly higher than that of the control group. This effect had time effect and concentration effect. At the same time, under the action of Araceae water extract, a variety of chromosomal aberrations appeared in the root tip cells of Vicia faba L., indicating that the water-soluble allelopathy of Auricularia auricula destroyed the normal structure and function of the spindle filaments of Vicia faba root tip cells. The formation of bicycles and the absence of centromere chromosome fragments formed a bridge, the formation of all bridges associated with the emergence of chromosome fragments [20]; due to individual chromosomes damaged or abnormal activity, resulting in chromosome retention phenomenon [20]. In addition, water-soluble allelopathy may interfere with the normal repair of DNA damage, resulting in the formation of chromosome fragments and lagged chromosomes cannot be polar motion, then in the nuclear reconstruction, the formation of the nucleus of the nucleus was from the main nucleus.

It can be seen that the water-soluble allelopathic substance has a certain genetic damage effect. It is presumed that the allelopathy of Solidago may be one of the mechanisms of its successful invasion and rapid diffusion.

5. Conclusion

The water-soluble allelochemicals of Solidago canadensis reduce the mitotic index of the crop, raise the micronucleus rate of the crop and induce all kinds of chromosomal aberrations, and have certain genetic toxicity. It is presumed that the allelopathy of Solidago can be successfully invaded and one of the mechanisms of rapid diffusion. It can be proved that the micronucleus test of Vicia faba root tip cells has great application value in detecting the allelopathy of invasive plants.

REFERENCES

[1] Li Xiaolan, Qiu Mingyi, Liu Supeng. Chemical constituents, pharmacological activity and clinical application of Solidago canadensis [J]. Journal of Traditional Chinese Medicine].

[2] Wang Yulan. Analysis of pharmacological effects of Chinese traditional medicine [J]. Chinese Journal of Folk Medicine, 2010, 03:31

[3] Zou Zhiming, Zhou Qingping, Ying Huifang, et al. Analysis of Phyllostachys edulis by infrared spectroscopy [J]. Journal of Traditional Chinese Medicine. 2007,18 (2): 312-313.

[4] Liu Supeng, Li Xiaolan, Qiu Mingyi, et al. Effects of Total Saponins of Total Flavonoids of Solidago canadensis on the Movement of Intestinal Smooth Muscle in Animals [J] .A Chinese Journal of Traditional Chinese Medicine, 2009, 30 (7): 926-92.

[5] Hierro J.L., Callaway R.M .. Allelopathy and exotic plant invasion [J]. Lant Soil. 2003, 256 (1): 29-39.

[6] Chen Ying, Shen Laihua, Cai Xianli, et al. A preliminary study on the allelopathy of two species of Rhizoma genus [J]. Wuyi Science. 2006, 22 (1): 176-181.

[7] Wang Kaijin, Li Ning, Chen Lizhong, et al. Chemical composition and antibacterial activity of essential oil of Solidago canadensis [J]. Journal of Plant Resources and Environment. 2006-01-25

[8] Shen Lihua. Studies on the allelopathic mechanism of alien plants in Solidago canadensis [J]. Fujian Agriculture and Forestry University 2007-04-01.

[9] SHAOH, PENGSI, WEIXY, et al. Potential allelochemical from an invasive weed Mikana micrantha H.B.K [J]. Journal of chemical Ecology, 2005, 31 (7): 1657-1667.

[10] Zeng R. A summary of biometric methods in the study of allelopathy. Chinese Journal of Applied Ecology, 1999, 10 (1): 123-126. [J]. Chinese Journal of Applied Ecology, 1999, 10 (1): 123-126.

[11] Chen Liping, Cao Mulan, Ma Danwei. A preliminary study on the toxicity of invasive plant. Journal of Sichuan Normal University (Natural Science Edition). 2008, 31 (3): 372-375.

[12] Du Fengchuan, Yu Shuhua, Ma Danwei, et al. Study on the Genetic Toxicity of Bidens pilosa to Root Tip of Vicia faba L. [J] .Acta Ecologica. 2007, l6 (3): 944-949.

[13] Zhou Xiaokui, Ma Dan, Zhou Quanquan, et al. Preliminary study on the genetic toxicity of invasive plant Mirabilis japonicus [J]. Journal of Southwest China Agricultural University, 2008, 21 (1): 152-155.

[14] Fan Xuetao, Ma Danwei, Liu Shuang, etc. Vicia faba root tip micronucleus test in spicy grass allelopathy research application [J]. Ecological Environment .2008,17 (1): 323-326.

[15] Gao P, et al. Acute toxicity and genetic toxicity of extracts. Journal of Anhui Agricultural Sciences. 2011, 39 (12): 7074-7075

[16] Tian Y, et al. Study on genetic toxicity and anti-mutagenic effects of water extract of Juglans. Cancer, Distortion, Mutation, 2011, 23 (3): 216-218

[17] Li Yaxuan, Zhao Xin. Genetics comprehensive experiment [M]. Beijing: Science Press, 2006: 19-23.

[18] Williamson G.B., Richardson D. Bioassays for allelopathy: measuring treatment responses with independent controls [J]. J Chem Ecol, 1988, 14 (1): 181-187.

[19] Zhao Fu Geng, He Longfei, Luo Qingyun. Plant Stress Physiological Ecology [M]. Beijing: Chemical Press, 2004: 25.

[20] Qian Xiaowei. Teratogenic effect of copper acetate on root tip cells of Vicia faba L. [J]. Journal of Cell Biology, 2005, 27: 351-357.