

Advances in Crop Improvement by Space Mutagenesis in China

Lu-xiang Liu¹, Hui-jun Guo, Linshu Zhao, Jiayu Gu and Shirong Zhao

¹The National Key Facility for Crop Gene Resources and Genetic Improvement, National Center of Crop Space Mutation Breeding, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Email: luxiang@263.net.cn

Abstract

Aerospace provides a special environment with strong cosmic radiation, microgravity, weak geomagnetic field and supervacuum, etc., which might affect plant growth and development as well as induce genetic changes of crop seeds. Since 1987, China has been conducting experiments of space mutagenesis for crop improvement by using recoverable satellites and *Shenzhou* spacecrafts. *Shijian-8*, the first world satellite specially designed for the space breeding program, was also launched on 9 September 2006. More than 40 new mutant varieties of crops including rice, wheat, cotton, sesame, pepper, tomato and alfalfa developed by space breeding have been officially released in China by far. A novel technique for mutation induction by simulating the space environment factors has also been set up. It was concluded that crop space-induced mutation breeding is an effective way to both breed new varieties and enhance genetic diversity.

Media summary

Exploration and application of space mutation induction by using recoverable satellite will promote crop germplasm enhancement and new variety development.

Key Words

Mutation, cosmic radiation, microgravity, germplasm, new variety, crop

Introduction

Plant mutation induction as an effective breeding approach in China has experienced more than fifty years. According to incomplete statistics by 2007, the total number of mutant varieties and mutant derived varieties officially registered in China accumulates to 741 including 45 crops and ornamental species (Liu et al. 2007a; 2007c). The popularization and utilization of all these good mutant varieties have made important contribution to China's food production and social and economic development. Mutation technique has become one of the most fruitful and widely used methods for crop improvement in China (Liu et al. 2004b). In the last twenty years, exploration and development of new mutagens such as space mutagenesis have been active to upgrade mutagenic efficiency in crop breeding. This paper briefly reviewed the recent advances and future perspectives of space-induced mutagenesis for crop improvement.

Space-induced mutation technique

The main characteristics of the aerospace environment that is far away from the earth are strong cosmic radiation, microgravity, weak geomagnetic field, supervacuum and superclean, etc. A large amount of

experimental research showed that space environment affected seed germination and growth of plants (Dutcher et al. 1994; Liu and Zheng 1997). The mitotic index declined remarkably. The type and frequency of chromosomal aberrations in space-flown seeds were greatly increased than that of the ground control. These mutagenic effects were universal in many plants. Results from space flight experiments showed that space conditions could be mutagenic (Halsted and Dutcher 1987; Mei et al. 1998).

The space-induced mutation technique, so called space breeding is a crop breeding technique which uses the good variations of plants (seeds) induced in the space environment that can be reached by the recoverable spacecraft (such as recoverable satellites and space shuttles) and high altitude balloon to choose new germplasms and new materials on the ground, then to develop new crop varieties (Liu et al. 2000).

Biological effects of space environment factors on plant seeds

The sensitivity to space flight differed between different species or different varieties in the same species of plants. After space flight and later germination on the ground, the seed vigor enhanced and the germination rate remarkably increased in seeds of wheat, triticale, barley, maize, cotton, sunflower, soybean, cucumber and tomato. There was no significant difference in germination rate compared with the ground control in seeds of rice, millet, pea, sweet pepper, lettuce and tobacco, while the seed germination was checked and germination rate decreased in seeds of sorghum, watermelon, eggplant, radish and towel gourd (Dutcher et al. 1994; Liu and Zheng 1997).

After being carried for space flight, the seed germination potential, germination index, seedling height and seedling vigor index of wheat, barley and triticale were all significantly higher than that of the ground control and gamma ray irradiation treatment. Such difference was fully proved in zymograms and activities of peroxidase isozyme and esterase isozyme, too. But the germination of sorghum seeds and seedling growth was strongly checked after space flight. Meanwhile its growth period also delayed. The growth habit and mature period of soybean were obviously affected after the seeds came back from space (Liu et al. 2004a)

Compared with the traditional gamma rays irradiation and other treatments, the greatest difference of agronomy performances in the first generation (SP1) of space-induced seeds was its reduced damage effect and that it could even possess stimulating effect on growth. In SP2 generation of dry seeds of variety Zhongzuo 59 of japonica rice carried by a high altitude balloon, all the 11 investigated characters which were plant height, growth period duration, spike length, grain husk color and light sensitivity, etc. segregated greatly. Especially some high quality rice types were selected and they could easily stabilize in later generations (Chen et al. 1994). Although the total variation frequency of space-induced mutation in SP1 generation in wheat was lower than that of the gamma rays irradiation treatment, it had more chance to generate good variations in SP1 generation by space treatment, with the variation frequency from 2.22% to 11.11% (Liu et al. 1996).

Mutant varieties developed by space breeding

Since 1987, China has conducted experiments of space-induced mutagenesis for plant improvement 21 times by using recoverable satellites, Shenzhou spacecrafts and high altitude balloons to carry plant seeds into space. Seeds were planted after returning from space flight for selection of useful mutations. Shijian-8, the breeding satellite specially designed for the space breeding program, was launched on 9 September 2006. It carried over 2000 accessions of plant seeds belonging to 133 species (Liu et al. 2007c).

So far China has officially approved 41 new varieties of crops including rice (Hangyu1, Huahang1, Eryouhang1, Teyouhang1, Yuehang1, Zhe101, Yuyou1, Teyou175 and Huaxiang7, etc.), wheat (Taikong5, Taikong6, Longfumai15, Hangmai96, Longfumai17 and Luyuan301), cotton (Zhongmiansuo42 and Zhongmiansuo52), sesame (Zhongzhi11 and Zhongzhi13), pepper (Yujiao1, Yujiao2, Yujiao3, Yujiao4 and Longjiao9), tomato (Yufan1 and Yufan2) and alfalfa (Longjing1) developed by space breeding technique. These new varieties have characters of high yield, good quality and multiple resistances. Some useful rare mutations that might make a great breakthrough in crop yield were also obtained (Qiu et al. 1998; Shi et al.2000; Liu et al. 2005a). Space breeding has begun to reveal good social and economic benefits.

It was showed that crop space-induced mutation breeding can be as a novel effective way to both breed new varieties and create distinctive genetic resources because of its wide mutation spectrum, high frequency of useful genetic variation as well as short breeding period.

Mechanism of space mutagenesis

The reasons why space environment could cause plant and chromosomal aberrations and then resulted in alterations of genetic characters were not very clear at present. Space radiation is one possibility. More multiple chromosomal aberrations were observed in seeds hit by HZE particles of cosmic radiation in space, and the abnormal rate of plant development increased. The aberration situations were different if HZE particles hit different parts of seeds. The frequency of aberration was the highest when the root meristem or hypocotyl was hit. But many experimental results showed that similar aberration increases could also be found in seeds not hit by cosmic particles in flight. The longer seeds were kept in space, the higher the frequency of aberration was, implying that microgravity had mutagenic effect on seeds, too (Halsted and Dutcher 1987; Gu et al. 1989).

Some newly researches on the effect of space environment suggested that space is effective in inducing the changes of crop genome. The genomic polymorphism in 201 rice plants developed from space flown seeds were investigated with RAPD analysis and found 30.2% of the polymorphism compared with plants from ground control seeds (Luo et al. 2006)

It was evident that the combined effects of both cosmic radiation and microgravity were the main causes of plant genetic changes induced by space conditions. Once the plants (seeds) which have been growing under the effects of earth gravity for billions of years were in the microgravity environment of space, affected by various physical radiation factors at the same time, their heredity would inevitably be strongly influenced. In addition, the strong vibration and blast force associated with spacecraft launch and landing was the one of important reasons that could not be neglected to cause increased frequency of chromosomal aberrations (Liu and Zheng 1997).

Study on simulating space environment factors

Because of the big investment and good technological support, the chance of the space experiment is very limited. It is important to make ground simulation on space factors to conduct research work for revealing the mechanism of space-induced mutation and applying it for plant breeding.

Experiments were conducted to simulate the space environment factors by accelerator or geomagnetic-field

free space (Liu et al 2004). The mutagenic effects of various space factors on wheat and other crops were studied from various angles of particle biology and physical field biology. It has been proven that the space environment has significantly different biological effects and mutagenic effects from traditional gamma rays. A new technique and method of mutation breeding by simulating the space environment has been set up preliminarily.

Magnetic field-free space

Equipment of the magnetic field-free space (MF) is a large magnetic screening installation using two-layer magnetic screening structure combining with the coil compensation. Its magnetic strength is 20 nT, which is 4×10^{-4} of the magnetic strength of the earth's magnetic field.

Air-dried seeds of wheat, etc., were treated by MF at room temperature for a certain period of time (Liu et al. 2002). The results showed that the seed germination and seedling growth were obviously inhibited by over 180 days of MF treatment. The striking morphological features of the wheat seedlings treated by MF were that the first leaf of the seedling turned shorter, wider and thicker; leaf apex turned blunt round and leaf color turned dark green. The MF treatment in the process of anther callus induction of wheat could stimulate the development of male gamete and final formation of calli, resulting in producing high quality anther calli and high percentage of green plants. These significant differences from the traditional γ -ray treatment showed that the MF had obvious mutagenic effect on wheat seeds and it could be used as a new physical mutagen other than γ -ray for wheat improvement. Some new mutant varieties have been developed by MF treatment in rice and alfalfa (Yu et al. 2006; Zhang et al. 2006).

Single high-energy ion beam implantation

Air-drying seeds of the wheat cultivars were irradiated by using single heavy ^7Li ion beam generated by tandem accelerator at the energy of 42.3MeV with different dosages. Biological effects studies showed that ^7Li ion beam implantation inhibited the germination rate, seedling height and root length in M1 generation. There was no linear dose-effect relationship but the "Braag peak" effect in ^7Li ion beam implantation with comparison of that of traditional gamma rays radiation. It was found 50 Gy could be the proper dose of ^7Li ion beam to irradiate wheat seeds at the energy of 42.3MeV. Compared with the gamma rays radiation, ^7Li ion beam implantation induced relatively lighter biological damage effects. Various morphological and cytological aberrations of the seedlings occurred. The most significant variations were chlorophyll deficiency of main vein, leaf split, leaf curl and tufted seedling (Wang et al. 2003; Liu et al. 2007d; Xin et al. 2007).

It was found that ^7Li ion beam implantation into crop seeds can produce the effects of not only the energy transferring, mass deposition and charge exchange as the same as the other ion beams, but also the reaction of $^1\text{H}(^7\text{Li}, ^7\text{Be})n$ in the irradiated seeds. Above results showed 50 Gy could be the proper dose of ^7Li ion beam to irradiate wheat seeds at the energy of 42.3MeV (Liu et al. 2007d).

Analysis of mutation types and frequencies of M2 populations showed that ^7Li ion beams could induce significant wheat mutations. Mutation phenotypes of ^7Li heavy ion beams were mainly spike types and plant height. Since the very limited M2 population size of ^7Li heavy ion treatments, the statistics of frequency of mutations and mutants was incomplete. However, the results that 50 Gy of ^7Li heavy ion treatment could produce the highest mutation in both frequency and types further revealed that 50 Gy could be the optimal irradiation dose for ^7Li heavy ion beam treatment in wheat (Guo et al. 2007; Liu et al. 2007b).

Mixed high-energy particles

Dry seeds of genotypes of winter wheat and alfalfa were irradiated by mixed high-energy particle field with different doses, which was generated from E2 beam lines of LINAC of Beijing Electron Positron Collider (Liu et al. 2004a; Liu et al. 2005b). The cytological effects on root tip cell of wheat seeds were studied with the same dosage of γ -rays irradiation as a control. The results showed that the irradiation of mixed high-energy particle field inhibited mitoses and produced various chromosomal aberrations such as micronucleus, chromosome bridges, circular chromosomes and dissociative chromosomes in root tip cells of wheat with significant dose-effects. Higher rate of cytological damages and percentage of chromosome circles and fragments were found in the mixed particle field irradiation than in γ -rays irradiation, indicating in the relative higher biological effects of irradiation of mixed high-energy particle field than those of γ -rays in the M1 generation of wheat (Han et al. 2006; Liu et al. 2005c)

Wider mutation spectrum and higher mutation frequencies as well as useful mutation frequencies for earlier maturity, shortness and spike type were observed in the M2 generation by mixed particle field irradiation than in that by γ -rays treatment. Mixed particle field could be used as a new mutagen to mutation induction and crop improvement (Liu et al. 2006)

Conclusions and prospects

Space-induced mutation technique is an effectively new way not only to develop new crop variety, but also possible to obtain rare mutants that may make a great breakthrough in important economic characters of crop, such as yield and quality, which are difficult to get using the other breeding methods on ground.

The research on applied basis of space-induced mutation technique needs to be strengthened. It seems very necessary to take further steps to work on those problems in space-induced mutation technique, such as material selection, treatment methods, molecular screening of mutants, earlier generation identification of quality characters, etc.

Because of the big investment and good technological support, the chance of the space experiment is very limited. It is important to make ground simulation on space environment factors to conduct research work for revealing the mechanism of space-induced mutation and applying it for crop breeding.

Newly developed biotechnological tools facilitating selection, characterization and genetic analysis of desired traits have significantly stimulated the use of space mutation induction in breeding and basic research. It is clear that the new development of space breeding will be heavily on and associated with not only effective use of the approach, but also advanced plant biotechnology, particular plant molecular biology. Therefore, international cooperation will be very important for the sustainable progress of the research related to the areas.

Acknowledgements

Research was successively supported by NSFC (39870192, 30270345 and 30670488), National Space Breeding Project (2003-138), National 863 Project(2002AA241011) and the International Atomic Energy Agency, Vienna through the Projects IAEA/RAS5040, RC12610, RC14195 and CPR5017.

References

- Chen Fangyuan, Jiang Xingcun, Lu Shengan, Yi Xiaoping, Yang Cunyi and Li Jinguo. 1994. Effect on rice genetical character by high space condition. *Chinese Journal of Rice Science* 8(1):1-8.
- Dutcher FR, Hess E and Halstead TW. 1994. Progress in plant research in space. *Adv Space Res.* 14: 159-171.
- Gu Ruiqi, Shen Huiming. 1989. Effects of space flight on the growth and some cytological characteristics of wheat seedlings. *Acta Photophysiological Sinica.* 15(4):403-407.
- Guo HJ, LX. Liu, WB. Han, SR. Zhao, LS. Zhao, L Sui, K Zhao, FQ Kong and J Wang. 2007. Biological effects of high energy ^7Li ion beams implantation on wheat. *Plant Mutation Reports.* 1(3):31-35.
- Halstead, TW and Dutcher FR. 1987. Plants in space, *Ann. Rev. Plant Physiol.* 38:317-345.
- Han W B, Liu L X, Guo H J, Zhao L S, Li J C, Zhang S P, Wang C S, Zhao S R.. 2006. SSR analysis on M1 variation of wheat irradiated by mixed high energy particle field. *Acta Agriculture Nucleatae Sinica*, 20: 165-168.
- Qiu Fang, Li Jinguo, Weng Manli, Jin Demin, Gao Hongyu, Wang Peisheng, Jiang Xingcun and Wang Bin. 1998. Molecular analysis of long-pod mutant line of mungbean generated by space mutagenesis. *Scientia Agricultura Sinica* 31(6):38-43.
- Liu Fuxia, Cao Moju , Rong Tingzhao , Pan Guangtang. 2005a. Screening a RAPD marker related to the maize male sterility gene obtained by space flight. *Journal of Sichuan Agricultural University* . 23(1): 19-23.
- Liu L X, Wang J, Zhao L S, Yang J C, Guo H J, Zhao S R, Zheng Q C. 2004a. Research progress in mutational effects of aerospace on crop and ground simulation on aerospace environment factors. *Acta Agriculture Nucleatae Sinica*, 18(4): 247-251.
- Liu L X, Zhao L S, Guo H J, Li J C, Wang J, Zhao S R, Zheng Q C. 2005b. Study on mutagenic effects of mixed particle field irradiation in winter wheat. *Science technology and engineering*, 21: 1642-1645.
- Liu L., L. Van Zanten, Q.Y. Shu and M. Maluszynski. 2004b. Officially released mutant varieties in china. *Mutation Breeding Review.* 14: 1-62.
- Liu Luxiang Wang Jing and Zheng Qicheng. 2000. Crop germplasm enhancement through aerospace mutation technique, In: *Proceedings of International Conference on Engineering and Technological Sciences* (Eds by Song Jian and Wang Maohua), 356-357, New World Press.
- Liu Luxiang, Guo Huijun , Zhao Linshu and Zhao Shirong. 2007a. Advances in induced mutations for crop improvement in China. in: *Proceeding of China-Korea Joint Symposium on Nuclear Technique Application in Agriculture and Life Science*, 22-25 April 2007, Hangzhou, China. 134-142.

- Liu Luxiang, Guo Huijun, Zhao Linshu and Zhao Kui. 2007b. Analysis of ^7Li ion beam induced DNA variations in wheat and plasmid pUC18, in: Annual report for Beijing National Tandem Accelerator Laboratory 2006, Beijing: Atomic Energy Press, 132-133.
- Liu Luxiang, Guo Huijun, Zhao Linshu, Gu Jiayu and Zhao Shirong. 2007c. Achievements in the past twenty years and perspective outlook of crop space breeding in China. *Journal of Nuclear Agricultural Sciences*. 21(6): 589-592.
- Liu Luxiang, Guo Huijun, Zhao Linshu, Zhao Kui, Zhao Shirong, Sui Li, Kong Fuquan. 2007d. Bio-effects of ^7Li ion beams irradiation on wheat. Annual report for Beijing National Tandem Accelerator Laboratory 2005. Beijing Atomic Energy Press, 104-105.
- Liu Luxiang, Zhao Linshu, Wang Jing Zheng Qicheng and Zhao Shirong. 2002. Biological effects of magnetic field-free space on wheat. *Acta Agriculturae Nucleatae Sinica*, 16(1):2-7.
- Liu Luxiang, Han Weibo, Guo Huijun, Zhao Linshu, Zhao Shirong, Li Jiakai, Wang Jin, Zheng Qicheng. 2005c. Cytological effects of mixed particle field irradiation in wheat. *Acta Agriculturae Nucleatae Sinica*, 19(5): 327-331.
- Liu Luxiang. and Qicheng Zheng. 1997. Space-induced mutations for crop improvement. China Nucl. Sci. & Tech. Report, CNIC01139/CSNAS-0111. China Nucl. Information Centre, Atomic Energy Press, Beijing.
- Liu Shuyu, Li Xinhua, Jin Liling, Wang Wenmei, Wun Guizhi, Gao Guoqiang and Dai Qun. 1996. The biological effects of the near-ground space treatment on M1 of wheat varieties. *Acta Agriculturae Nucleatae Sinica* 10(4):205-210.
- Luo Yi, Wang Xujie, Mei Mantong, Zhuang Chuxiong, Zhou Feng, Wei Zengquan, Xie Hongmei, Yao Juan and Zhao Junliang. 2006. Genomic polymorphism in consecutive generation rice plants from seeds on board spaceship and their relationship with space HZE particles, *Acta Biophysica Sinica* 22 (2): 131-137.
- Mei M, Qin Y and Sun Y. 1998. Morphological and molecular changes of maize plants after seeds been flown on recoverable satellite. *Advances in Space Research*. 22: 1691-1697.
- Shi Jinguo, Fan Qingxia, Wang Linqing, Hu Ping, Sun Guoqing, Li Guiying. 2000. Induction of large grain mutation in adzuki bean (*Phaseolus angularis* Wight) by space environmental condition. 14(2):93-98.
- Wang J, Liu L X, Zhao S R, Yang J C, Guo H J, Zhao L S, Chen W H. 2003. Preliminary study on biological effects of ^7Li ion beams on *Echinacea purpurea*. *Acta Agriculture Nucleatae Sinica*, 17: 405-408.
- Xin Qingguo, Liu Luxiang, Guo Huijun, Zhao Linshu, Yu Yuanjie, Zhao Kui, Sui Li, Kong Fuquan and Zhao Shirong. 2007. SSR analysis of M1 variation of dry seeds implanted by ^7Li ion beam in wheat. *Journal of Triticeae Crops*. 27(4):560-564.
- Yu Qiucheng, Liu Luxiang, Huang Baocai, Xu Guozhan, Zhang Zhi-ing. 2006. Selection of high quality male sterility line of indica rice by field-free magnetic space induction and its characters. *Acta Agriculture Nucleatae Sinica*. 20(6):497-499.

Zhang Yuexue, Tang Fenglan, Zhang Hongqiang, Han Weibo, Li Daoming, Liu Jielin, Hao Ruochao, Shen Zhongbao and Liu Luxiang. 2006. Breeding of new variety nongjing no.1 of medicago sativa l. by magnetic field free space. Journal of Nuclear Agricultural Sciences. 21(1):34-37.