Plant Omics Journal POJ

POJ 6(5):355-358 (2013) ISSN:1836-3644

Research Note

Mutagenic effects of low energy ions on root tip cells of tomato (*Lycopersicum esculentum*)

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Abstract

Low energy ion has been successfully applied to crop breeding, but there are few studies on application of ion beam in tomato breeding. In this research, effects of ion beam on root tip cells of tomato were studied. We found that nitrogen ion (N^+) or argon ion (Ar^+) had obvious influences on cell mitosis and chromosome structure, and lead to various types of chromosome aberration. When dose of ion beam increased within the scope of 1-4×10^{17} ions cm^{-2}, the index of cell mitosis declined and rate of chromosome aberration increased, and various types of chromosome aberration occurred in the same cell when dose of ion beam was high. In addition, compared to the same dose of Ar^+ ion, the index of cell mitosis was lower and rate of chromosome aberration was higher as implanted with N^+ ion, suggesting N^+ ion had greater influence on cell mitosis and chromosome structure. Taken together, cell aberration could be induced by N^+ or Ar^+ ion, and genetic material of tomato might be damaged, therefore low energy ion is potential to be as mutagenic agent in breeding and improvement of tomato.

Keywords: Cell mitosis; chromosomal aberration; Ar^+ ion; N^+ ion; tomato.
Abbreviations: Ar^+_argon ion; N^+_nitrogen ion.

Introduction

Low energy ion beam biotechnology was firstly initiated by Yu in the 1980s (Yu et al., 1989), and has many advantages, such as simple and reliable method, a variety of ions, lower cost, short breeding time, and so on (Zhou, 2009; Cao et al., 2011). Besides having common characteristics of heavy ion, when low energy ion is implanted into organism, energy deposition, momentum transmission and mass deposition would occur and collectively act on organism, subsequently lead to variation of genetic material and initiate biological effects (Du et al., 2000; Huang and Chen, 2006; Kikuchi et al., 2009; Jie et al., 2010), which could be novel and practical in formation of genetic variations. At present, low energy ion has been successfully applied to some crop breeding, such as rice, wheat, soybean, tobacco, cotton, rape and others (Zhou, 2009). Thus, low energy ion might provide one new way for genetic modification; however, function mechanism of low energy ion is very complicated and need to be further explored.

Tomato is annual herb plant and generally grows around the world. It is well known; tomato contains abundant nutrients, such as lycopene, vitamin C, protein, fat, trace elements and other nutrients, appears higher nutritional value, and is named the first in ten kinds of most potent food by Time Magazine. In order to meet needs of market and people, breeding some tomato cultivars with distinctive characters is a task which brooks no delay. Compared with other breeding methods, ion beam mutation breeding technology is characterized as limited physiological damages, high mutation frequency, wide mutation spectrum, and so forth (Ji et al., 2005), but research on application of ion beam in tomato breeding has been still less. In this study, dry seeds of tomato were irradiated with different doses of N^+ or Ar^+ ion, cell mitosis and chromosome aberration in root tip cells of tomato were studied, which would provide basis for mutagenic mechanism of low energy ion and application of low energy ion in tomato breeding.

Results and discussion

Effects of ion beam on cell mitosis

When organism is irradiated and implanted by ion beam, a series of responses would happen, such as physical reaction, chemical reaction, and so on, which would have certain damage on organism, even destroy structure and function of genetic material, then could disturb biochemical and physiological processes of cells (Jie et al., 2010). In this study, cell mitosis in root tip of tomato was obviously influenced by ion beam. As listed in Table 1, compared with CK, the index of cell mitosis was on the decline along with dose increase of N^+ ion, was respectively 9.29%, 8.43%, 7.71% and 6.77%, in which effects of 3×10^{17} N^+ cm^{-2} and 4×10^{17} N^+ cm^{-2} on index of cell mitosis reached extremely significant differences ($P \leq 0.01$). Similarly, when dose of Ar^+ ion increased, the index of cell mitosis also tapered, in order, 9.34%, 8.67%, 8.11% and 7.41%, and effects of 3×$10^{17}Ar^+$ cm^{-2} and 4×$10^{17}Ar^+$ cm^{-2} on index of cell mitosis also took on extremely significant differences ($P \leq 0.01$). In short, the number of mitotic cells both declined in root tip of tomato implanted with N^+ or Ar^+ ion, furthermore the index of cell mitosis had a negative relationship with dose of ion beam, which is in accord with research results found by Du et al. (2000) and Wu et al. (2010). Thus, we infer that sputtering and etch of ion beam would cause damage to cell and influence cell mitosis, even lead to genetic variations (Jie et al., 2010). In addition, under the same dose of ion beam, the

Table 1. The index of cell mitosis in root tip of tomato implanted with ion beam.

Ion	Implantation dose	Index of cell mitosis (%)
CK	0.00	12.02
N^+	1×10^{17} N^+ cm^{-2}	9.29*
	2×10^{17} N^+ cm^{-2}	8.43*
	3×10^{17} N^+ cm^{-2}	7.71**
	4×10^{17} N^+ cm^{-2}	6.77**
Ar^+	1×10^{17} Ar^+ cm^{-2}	9.34*
	2×10^{17} Ar^+ cm^{-2}	8.67*
	3×10^{17} Ar^+ cm^{-2}	8.11**
	4×10^{17} Ar^+ cm^{-2}	7.41**

Note: * and ** separately stand for significant difference ($P\leq0.05$) or extremely significant difference ($P\leq0.01$).

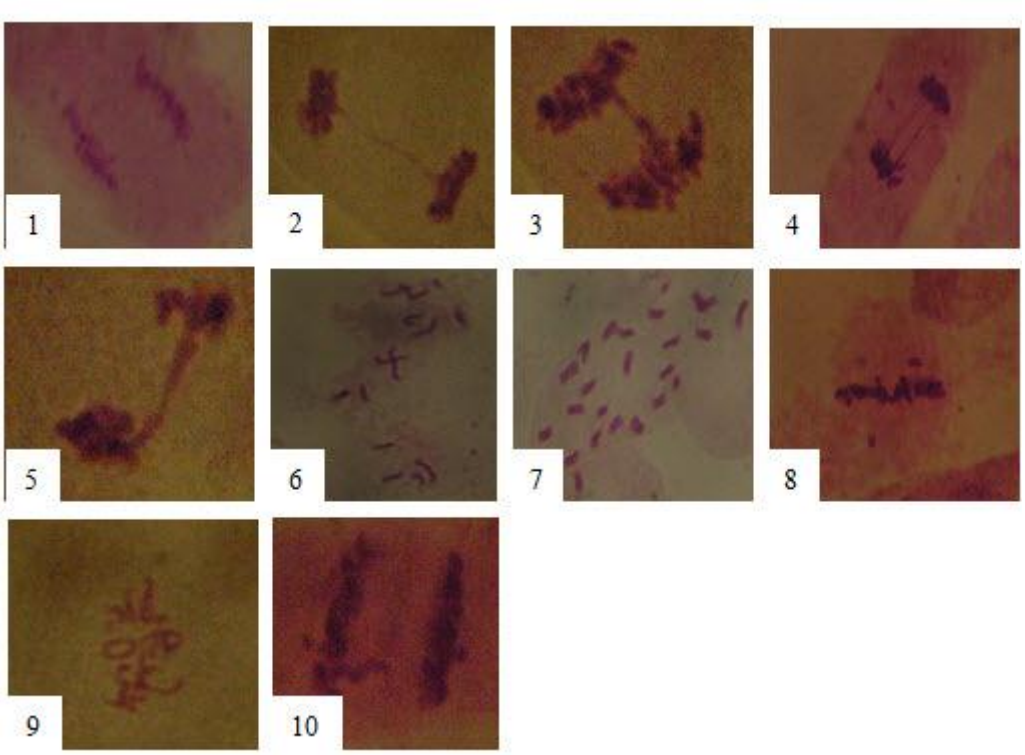

Fig 1. Chromosome aberration in root tip cells of tomato implanted with N^+ or Ar^+ ion beam. 1. Anaphase in normal mitosis; 2-3. Chromosome single bridge; 4. Chromosome twin bridge; 5. Chromosome single bridge and free chromosome; 6. Free chromosome; 7. Normal chromosome; 8. Chromosome fragment ; 9. Chromosome adhesion; 10. Lagging chromosome.

index of cell mitosis in root tip of tomato irradiated by N^+ ion was lower than that by Ar^+ ion (Table 1). As listed in Table 1, when tomato seeds were implanted with 2×10^{17} ions cm^{-2}, the index of cell mitosis in root tip of tomato exposed to N^+ ion or Ar^+ ion was respectively 8.43%, 8.67%, and was 6.77% or 7.41% accordingly as implanted with 4×10^{17} ions cm^{-2}, which suggests the inhibitory action of N^+ ion on cell might be stronger.

Effects of ion beam on chromosome structure

As shown in many studies, ion beam could bring about chromosome aberration, and various kinds of chromosome aberration were found, such as chromosome adhesion, chromosome fragment, lagging chromosome, chromosome single bridge, chromosome multiple bridges, free chromosome and others (Lin et al., 1991; Wu and Yu, 2001; Hase et al., 2002; Hou et al., 2008; Dai et al., 2011). Moreover, deficiency, repetition or recombination of gene and other genetic effects might occur because of chromosome aberration (Hu et al., 1994). In this research, chromosome single bridge, chromosome twin bridges, lagging chromosome, free chromosome, chromosome fragment and other chromosome aberration were also found in root tip cells of tomato implanted with N^+ or Ar^+ ion beam (Fig 1). Statistical analysis revealed in Table 2, when dose of N^+ ion increased within the scope of $1\text{-}4\times10^{17}$ N^+ cm^{-2}, total rate of chromosome aberration increased, was respectively 1.20%, 1.95%, 2.57% or 3.37%, and rate of every chromosome aberration gradually increased, in which the main chromosome aberration was chromosome bridge, secondly was free chromosome, while lagging chromosome, chromosome fragment and chromosome adhesion were relatively less. In addition, when dose of Ar^+ ion beam increased within the scope of $1\text{-}4\times10^{17}$ Ar^+ cm^{-2}, total rate of chromosome aberration was gradually on the increase, was respectively 1.06%, 1.21%, 1.57% or 2.22%, and rate of every chromosome aberration also increased, the main

Table 2. Rate of chromosome aberration in root tip cells of tomato implanted with ion beam.

Ion beam and dose	Rate of chromosome aberration (%)					Rate of total aberration (%)
	Chromosome bridge	Free chromosome	Lagging chromosome	Chromosome fragment	Chromosome adhesion	
0.00 (CK)	0.00	0.00	0.00	0.00	0.00	0.00
1×10^{17} N^+ cm^{-2}	0.52	0.21	0.14	0.18	0.15	1.20
2×10^{17} N^+ cm^{-2}	0.94	0.32	0.23	0.25	0.21	1.95
3×10^{17} N^+ cm^{-2}	1.21	0.43	0.28	0.34	0.31	2.57
4×10^{17} N^+ cm^{-2}	1.64	0.61	0.35	0.41	0.36	3.37
1×10^{17} Ar^+ cm^{-2}	0.43	0.18	0.12	0.14	0.19	1.06
2×10^{17} Ar^+ cm^{-2}	0.54	0.16	0.15	0.16	0.20	1.21
3×10^{17} Ar^+ cm^{-2}	0.61	0.21	0.18	0.20	0.37	1.57
4×10^{17} Ar^+ cm^{-2}	0.98	0.34	0.25	0.24	0.41	2.22

chromosome aberration was also chromosome bridge, however other kinds of chromosome aberration were fewer. It was demonstrated that chromosome bridge generates mainly because chromosome with double centromeres fractures in metaphase of cell mitosis, which suggests chromosome damage caused by ion beam would influence each division phase in cell cycle (Wu et al., 1997). Wu and Yu (2001) also found that frequencies of mitotic cells with chromosome aberrations increased along with dose increase of N^+ ion, but chromosome aberrations in root tip cells of wheat mainly consisted of acentric fragments and deletions, and chromosome bridges was only the main aberration in pollen mother cells of wheat. Furthermore, as implanted with the same dose of ion in this study, the teratogenic capacity of N^+ ion on chromosome was more obvious than that of Ar^+ ion, especially rate of chromosome bridge was significantly higher (Table 2). Besides, many kinds of chromosome aberration would simultaneously exist in the same cell along with dose increase of ion beam.

Materials and Methods

Plant materials

In this study, seeds of tomato (the miscellaneous 9 variety) were kindly provided by Vegetable Flower Institute of Agricultural Sciences, Beijing, P. R. China.

Implantation of ion beam

The implantation of ion beam was performed in Key Lab of Ion beam Bioengineering in Zhengzhou University, Henan, P. R. China. Tomato seeds were evenly placed in TITAN ion implanter which is produced by Institute of High Voltage (Siberian Division of the Russian Academy of Science, Russia), and were respectively irradiated by N^+ or Ar^+ ion beam under 30kev energy conditions. The implantation dose of ion beam was 1×10^{17} ions cm^{-2}, 2×10^{17} ions cm^{-2}, 3×10^{17} ions cm^{-2} and 4×10^{17} ions cm^{-2}, furthermore 1000 tomato seeds were respectively irradiated with different doses of N^+ or Ar^+ ion beam.

Germination of tomato seed

Seeds implanted with N^+ or Ar^+ ion, and seeds of control group (CK) which were not irradiated by ion beam, had been firstly soaked into sterile water for 24h at 25℃, and were respectively placed in Petri dish at whose bottom there were two layers of filter papers, then were cultured at 25℃ under 14h photoperiod of 1,000-1,200 lux illumination intensity. In addition, the water in Petri dish was changed every day, and root tip of tomato was cut when the radicle was longer than length of seed.

Observation of cell division and chromosome

The root tip was washed with distilled water after treated for 3h in paradichlorobenzene solution, and transferred to Carnoy's solution for 24h, subsequently root tip was washed with distilled water, and then was preserved in 70% ethanol. The root tip preserved in 70% ethanol was used to observe cell division and chromosome structure, and the chromosome was prepared according to the following steps, dyeing with improved carbol fuchsine, chromosome tabletting, microscopic examination and photograph. In this study, about 2000 cells in each root tip were observed, and there were 5-10 root tips of tomato in each group. Furthermore, the number of mitotic cells, cells with chromosome aberration and types of chromosome aberration were respectively counted.

Data processing and analysis

SPSS 10.0 software was used to process and analyze test data, the computational formula about index of cell mitosis and rate of chromosome aberration was respectively described in the following: Index of cell mitosis (%) = (number of mitotic cells / number of cells observed) × 100%; Rate of chromosome aberration (%) = (number of cells with chromosome aberration / number of mitotic cells) × 100%.

Conclusion

The present study indicated that cell mitosis in root tip of tomato was evidently influenced by Ar^+ or N^+ ion beam, and index of cell mitosis reached extremely significant differences when dose of ion beam was $3\text{-}4\times10^{17}$ ions cm^{-2}. Simultaneously, many kinds of chromosome aberration were also found, and rate of chromosome aberration had a positive relationship with implantation dose of ion beam, especially chromosome bridge was the main chromosome aberration. Furthermore, effects of N^+ ion on cell mitosis and chromosome structure were greater than those of Ar^+ ion. In short, implantation of ion beam made cell mitosis decrease and influenced chromosome structure.

Acknowledgments

This research was kindly supported by Science Fund from Henan province (122300410025) and grant of young teachers in Henan province institution of higher learning (2011GGJS-063), in P. R. China.

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