# RAPD analysis of alfalfa DNA mutation via $N^+$ implantation

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**Abstract** Germination capacity of alfalfa seeds under low energy $N^+$ implantation manifests oscillations going down with dose strength. From analyzing alfalfa genome DNA under low energy $N^+$ implantation by RAPD (Random Amplified Polymorphous DNA), it is recommended that 30 polymorphic DNA fragments be amplified with 8 primers in total 100 primers, and fluorescence intensity of the identical DNA fragments amplified by RAPD is different between CK and treatments. Number of different polymorphic DNA fragments between treatment and CK via $N^+$ implantation manifests going up with dose strength.

**Keywords** Alfalfa, $N^+$ implantation, Germination capacity, RAPD (Random Amplified Polymorphous DNA)

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## 1 Introduction

Low energy ion implantation as a way of mutation breeding has been characterized by a higher mutation rate and wider mutation spectrum with lower damage to organism, therefore it has been widely applied to breeding crops and microbes since 1997, and better economic and social benefit has been acquired.[1] Concerning nature of the bioeffect induced by low energy ion, many researches were made about chromosome changes and DNA damage outside organism.[2-4] The influence induced by low energy ions on DNA molecules in living body is important to study nature of the bioeffect. In this paper, we use RAPD (Random Amplified Polymorphous DNA) to analyze mutation of alfalfa genome DNA induced by $N^+$, and discuss why germination capacity of alfalfa seeds under low energy $N^+$ implantation manifests oscillations going down with dose strength.

## 2 Materials and methods

Each treatment group is composed of 200 alfalfa seeds sterilized, which were placed on plate with their embryo facing the ion beam and set into bar room, and were implanted with $N^+$ beam. At the same time, seeds of CK group also were placed into bar room without implantation with $N^+$ beam. Then seeds were cultivated on agar culture medium at 26ºC. Germination capacity was calculated in a week and seedlings were transplanted onto 1/2MS culture medium. After 20 day cultivating, seedlings were cut out to extract genome DNA using Murry's method.[5] Procedure of RAPD reaction followed Yu Kang-Fu's method,[6] and RAPD reaction was repeated 3 times for every primer.

## 3 Results

According to results of many identical experiments, germination capacity of alfalfa seeds under $N^+$ implantation manifests oscillations going down with dose strength (Fig.1). Genome DNA extracted from leaves was analyzed by gel electrophoresis in 0.8% agarose. The photo of EB stained agorose gel has illustrated that genome DNA is characterized by better integrality. In this experiment, 100 primers that were recorded as $S_1$-$S_{100}$ were chosen for RAPD analysis of genome DNA extracted from seedlings of 5 treatments and CK. There are 30 polymorphic DNA fragments amplified using 8 primers, whose sequences are listed in Table 1. Number of different polymorphic DNA fragments between treatment and CK goes up with

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dose strength. In fluorescence intensity of identical DNA fragments amplified by RAPD exists difference between treatments and CK (Table 2).

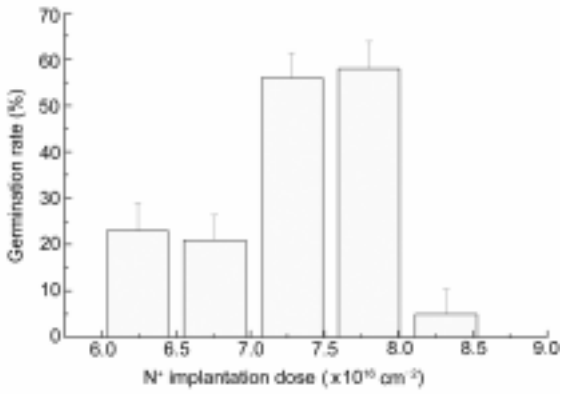


**Fig.1** Germination rates of alfalfa seeds under different doses of $N^+$ implantation.

**Table 1** Sequences of 36 primers

| S | Sequences | S | Sequences | S | Sequences |
|---|---|---|---|---|---|
| 41 | ACCGCGAAGG | 42 | GGACCCAACC | 45 | TGAGCGGACA |
| 46 | ACCTGAACGG | 50 | GGTCTACACC | 52 | CACCGTATCC |
| 56 | AGGGCGTAAG | 58 | GAGAGCCAAC | | |

**Table 2** The number of different polymorphic DNA fragments between treatment and CK

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| *N* | 3 | 3 | 4 | 5 | 15 |

*N*: the number of different polymorphic DNA fragments between treatment and CK; 1~5: treatment groups of alfafa implanted by $N^+$ at the dose of $6.24\times10^{16}$, $6.76\times10^{16}$, $7.28\times10^{16}$, $7.80\times10^{16}$ and $8.32\times10^{16}$ $cm^{-2}$, respectively.

## 4 Discussion

As for 30 polymorphic DNA fragments amplified, there are three possible explanations: (1) Bases of complementary sites with primers are changed in genome DNA, and these changes cause complementary sites decreasing or increasing, consequently, DNA fragments amplified decrease or increase; (2) DNA insertion or DNA absence happens between complementary sites, which cause change of molecular weight of DNA fragments amplified; (3) DNA fragments are inserted too large to be amplified. Difference in fluorescence intensity of RAPD products can be attributed to the fact that the base change of complementary sequence having many copies with primer makes RAPD products decrease with the primer, or that new DNA fragments amplified by RAPD have similar number of base pairs, which strengthens fluorescence intensity of RAPD products. It is recommended that there happen DNA insertion or absence or base change in genome DNA via $N^+$, furthermore, structure and function of DNA macromolecule be affected. Number of polymorphic DNA fragments increases dramatically from 5 to 15 and germination capacity reduces violently when the $N^+$ dose goes up from $7.80\times10^{16}$ $cm^{-2}$ to $8.32\times10^{16}$ $cm^{-2}$. It is deduced that genome DNA mutation under $N^+$ implantation at the dose of $8.32\times10^{16}$ $cm^{-2}$ be impossibly repaired, which brings about that seeds can not germinate. We can infer by above results of experiments that $N^+$ implantation can give rise to many base changes on genome DNA and base changes increase with dose strength; DNA change via $N^+$ implantation at lower dose lowers directly germination capacity, however, some mutation sites may be located on genes

about DNA repairing system and stimulate expression of genes or heighten activity of expression products, and function of DNA repairing system is enhanced, as a result that germination capacity of seeds rather rise than decrease successively under this dose scope. Further sites changed can not be repaired in time by DNA repairing system with dose further strengthened, or DNA repairing system can not execute function due to its own damage, consequently, germination capacity decreases dramatically.

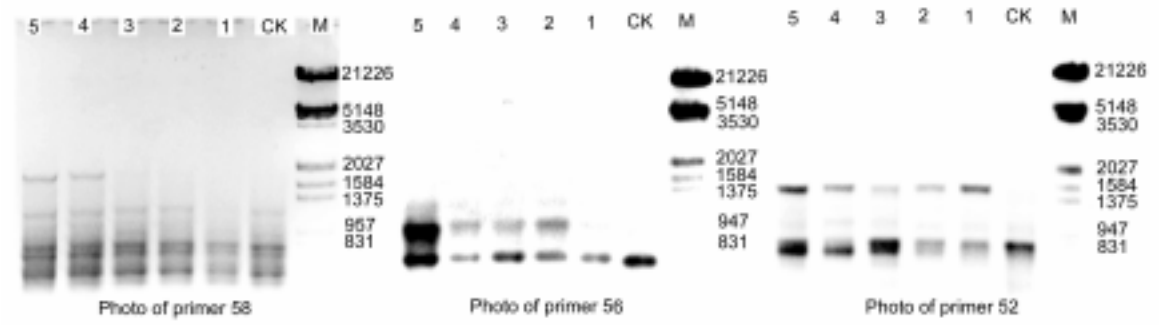


**Fig.2** Illustration of RAPD analysis of alfalfa genome DNA. M: marker; 1-5: treatment groups of alfalfa implanted by $N^+$ at the dose of $6.24\times10^{16}$, $6.76\times10^{16}$, $7.28\times10^{16}$, $7.80\times10^{16}$ and $8.32\times10^{16}$ $cm^{-2}$, respectively.

The survival rate via ion implantation manifests oscillations with dose strength, which has been also observed by other authors.[2,7] YU Zheng-Liang proposed that combination of energy absorption, mass deposition, and charge exchange of energetic ions in the organism results in the above-mentioned phenomenon (1). In this paper, the authors have also supposed an explanation of the phenomenon according to the result of genome DNA mutation sites increasing with dose strength. The problems of where the mutation sites located in the genome DNA, and what after-effect of the mutation on organism, will depend on further research.

## References

1 Yu Z L. IEEE Trans Plasma Sci, 2000, **1**(28): 128-132

2 Yu Z L. Introduction of Ion Beam Biotechnology (in Chinese), Anhui Science and Technology Press, 1998, 145

3 Wang X Q, Han J W, Yu Z L. Acta Biophys Sin (in Chinese), 1998, **14**(2): 337-340

4 Yang J B, Wu L J, Li L *et al*. Sci China C, 1997, **40**(1): 107-112

5 Murry M G, Thompson W F. Nucl Acid Res, 1980, **8**: 4321-4325

6 Yu K F, Peter Pauls K. Optimization of DNA-extraction and PCR procedures for random amplified polymorphic DNA (RAPD) analysis in plants, PCR Technology Current Innovation CRC Press, 1994, 193-200

7 Joiner M C, Marples B, Lambin P *et al*. Int J Radiat Oncol Biol Phys, 2001, **49**(2): 379-389