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Selection of disease resistant lines of *Rehmannia glutinosa* with mutant treatment

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ABSTRACT : ***Rehmannia glutinosa*** **is one of the most important medicinal crops in Korea. However, various plant pathogens including *Fusarium* sp. cause great damages and cause enormous economic losses. Therefore, this study was conducted to select *Fusarium* resistant plants by using mutagen-treated *Rehmannia glutinosa*. The plant material used was a native accession of *Rehmannia glutinosa*. As a result, among the *Rehmannia glutinosa* treated with various concentrations of EMS, *R. glutinosa* treated with 0. 03M EMS showed higher resistance against infection of *F. oxysporum* in pot tests, and *Rehmannia glutinosa* plants treated with 0.12M EMS showed higher resistance against infection of *F. oxysporum* in field tests.**

Key words : *Rehmannia glutinosa*, disease resistance, EMS, *F. oxysporum*

INTRODUCTION

Rehmannia glutinosa is a perennial medicinal plants belonging to the family Scrophulariaceae, and there are ca. 300 species known in the world, especially in temperate regions. Its roots have been used widely in Korea for a long time for medicinal purposes. *Rehmannia glutinosa* contains iridoid, catalpol, leonuride, stachyose, sucrose, mannitol, and amino acid (2), and it is used for hematic, robustness, cardiotonic drug, diabetes treatment, antifebriel, and detoxification purposes (1).

Rehmannia glutinosa shows weak fertility and it mainly proliferates through root branching and rootlet growth. However, roots for proliferation are usually infected with various pathogens during the storage, and these infections cause great damages to the roots and impedes the intensive farming of the crop. *Rehmannia glutinosa* can be infected with various pathogens in the field and shows various symptoms including leaf spots by *Phoma* sp., root rots by *R. solani* and *F. oxysporum*, *Fusarium* wilts by *F. solani*, and *Corticium* rot by *C. rolfsii*. Mosaic symptoms caused by various viruses can also be observed. Among these various pathogens, *F. oxysporum* caused the greatest damages in Korea in recent years (3, 4, 5, 6). Therefore, it is important to breed disease resistance against these pathogens with various methods. Research works on disease-resistant line selection of *Rehmannia glutinosa* with mutagen treatment is one of the methods used. This method was attempted on *R. glutinosa* previously (4, 5, 6). However, further research was needed, and, therefore, for the selection of resistant lines, *R. glutinosa* were treated with different concentrations of EMS and their resistance against *F. oxysporum* infections were tested in pot and field tests.

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MATERIALS AND METHODS

Induction of mutation with EMS treatment.

The plant material used was a native accession of *Rehmannia glutinosa*. The plant materials obtained were cultured *in vitro* according to the method reported previously (Huang et al., 1999). *In vitro* cultured roots (50g/l explant) of *R. glutinosa* were treated with Ethyl Methane Sulphonate(EMS) at concentrations of 0.03, 0.06, or 0.12M, and transplanted into pots for the induction of mutation three days after the treatment. EMS treated plantlets were grown for six months before their growth characteristics were analyzed.

Pathogenicity tests of EMS-treated plants in pots.

In vitro cultured and selected mutant plants were acclimatized in the green house and transplanted into pots before inoculation of the pathogen. Inoculums were prepared by culturing the *Fusarium oxysporum* isolates R-3 and R-10 in liquid PDA medium at 24℃ for 7 days. Mutation of *R. glutinosa* were verified with opa 3 primer, and selected mutant plant roots were wounded and inoculated with *Fusarium oxysporum* through dipping method. Pathogenicity tests were performed three times. Results of the pathogenicity tests were observed four weeks after the inoculation.

Pathogenicity tests of EMS-treated plants in field.

In vitro cultured and selected mutant plantlets were acclimatized in the green house, transplanted into the field, and grown for two months before inoculation of the pathogen *Fusarium oxysporum* isolate R-10. Inoculums were prepared by culturing the *Fusarium oxysporum* in liquid PDA medium at 24℃ for 7 days. Plant roots were inoculated with *Fusarium oxysporum* through dipping method. Pathogenicity tests were performed three times. Results of the pathogenicity tests were observed four weeks after the inoculation.

RESULTS AND DISCUSSION

Growth characteristics of EMS treated plants.

All the EMS treated plants showed increased values for all the parameters evaluated (Table 1). Compared to the control plants, all the EMS treated plants increased in shoot length and root length. Shoot and root fresh weights of EMS treated plants also increased dramatically compare to the control plants (Figure 1). Among the EMS treated plants, plants treated with 0.03M EMS showed the highest value in shoot fresh weight, root fresh weight, shoot length, and root length.

Table 1. Comparison of growth parameter of EMS treated plants[a]

Concentraion of EMS	Shoot fresh weight(g)	Root fresh weight(g)	Shoot length (cm)	Root length (cm)
Control	57.0	56.0	4.5	22.0
0.03M	102.3	91.3	5.7	26.9
0.06M	89.0	75.3	5.4	21.8
0.12M	86.5	70.0	5.0	25.1

[a]The results of each treatment were the mean of triple experiments and 100 plants were used for each experiment.

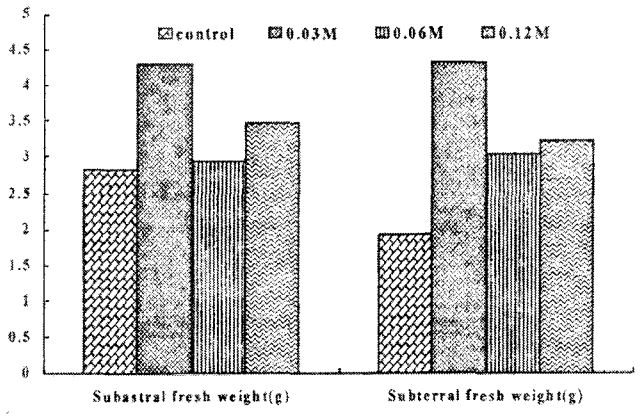


Fig. 1. Comparison of characteristic of EMS treated plant in pot.

Pathogenicity tests of EMS-treated plants in pots.

As a results of the pathogenicity tests in pots, all the control (EMS-untreated) plants showed medium susceptibility to isolate R-3 and high susceptibility to isolate R-10 of *F. oxysporum*. Plants treated with 0.06M and 0.12M EMS showed more severe symptoms

Table 2. Results of pathogenicity tests with mutant *Rehmannia glutinosa* inoculated with two different pathogenic isolates of *Fusarium oxysporum* in pots.

Mutagenic plant	*Fusarium oxysporum*	
	R-3	R-10
Control	1[a](0[b])	1(0)
	2(4)	2(0)
	3(13)	3(8)
	4(9)	4(4)
	5(4)	5(18)
0.03M	1(0)	1(4)
	2(21)	2(15)
	3(9)	3(4)
	4(0)	4(3)
	5(0)	5(4)
0.06M	1(2)	1(2)
	2(14)	2(9)
	3(10)	3(11)
	4(4)	4(4)
	5(0)	5(4)
0.12M	1(0)	1(0)
	2(2)	2(2)
	3(13)	3(8)
	4(5)	4(10)
	5(10)	5(10)

[a]Disease ratings in 1-5 scales; 1 = no disease observed and 5= death of plants.
[b]The number of plants showing the disease ratings indicated out of total 30 plants tested.

of wilts and root rots compared to those treated with 0.03M EMS. In R-10 inoculated plants, most untreated-plants showed disease severity of 3-5, and fifteen plants out of thirty 0.03M EMS treated plants showed mild symptoms. These results indicated the possible resistance of 0.03M EMS treated plants against the infection of both isolates of *F. oxysporum* (Table 2).

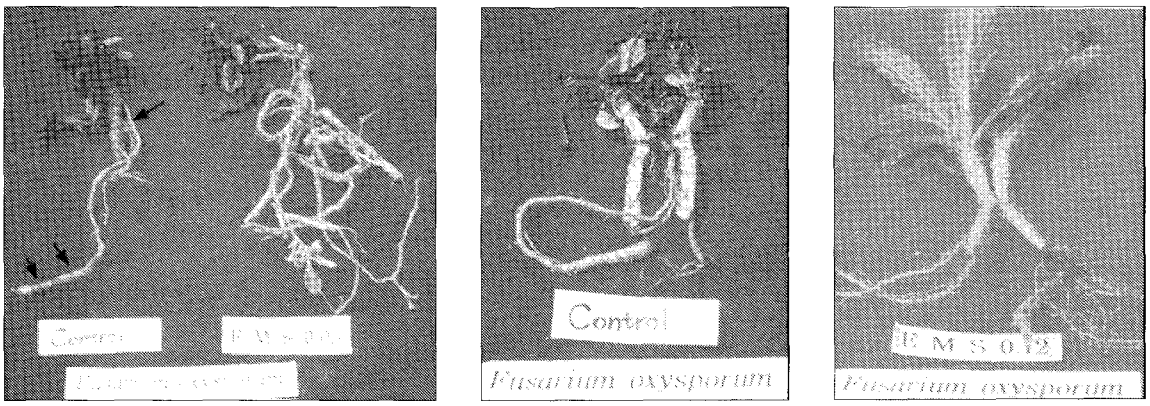
Pathogenicity tests of EMS-treated plants in fields.

Mutant treated *R. glutinosa* plants were transplanted and inoculated with R-10 isolate of *Fusarium oxysporum*, and the results were evaluated thirty days after the inoculation. As a result, eleven control (EMS-untreated) plants out of fifteen plants showed severe symptoms and high disease severity ratings of 3.9 (Table 3). On the other hand, plants treated with 0.03M, 0.06M, and 0.12M EMS showed disease ratings of 3.1, 3.3, and 2.2, respectively. In the field tests, plants treated with 0.12M showed higher resistance against the infection of R-10 compared to others. Few plants treated with 0.12M EMS showed severe symptoms of disease, and this, probably, resulted from selection pressures. Higher resistance in plants treated with higher concentrations of EMS might be explained with the fact that higher concentrations of EMS increase the biomass of roots. Therefore, further studies might be necessary for the selection of resistant lines.

Table 3. Results of pathogenicity tests with mutant *Rehmannia glutinosa* inoculated with pathogenic isolate (R-10) of *Fusarium oxysporum* in field tests.

	Fusarium oxysporum R-10															
Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Average
Control	4	3	4	5	2	4	4	5	4	5	4	3	4	5	4	3.9
0.03M	5	3	3	3	3	3	2	2	3	3	3	4	3	4	3	3.1
0.06M	3	5	3	4	3	2	3	4	2	3	3	4	4	3	2	3.3
0.12M	1	1	2	2	3	4	3	3	2	1	1	2	3	1	2	2.2

[a]Disease ratings in 1-5 scales; 1 = no disease observed and 5=death of plants.



(A) (B) (C)

Fig. 2. Pathogenicity test of mutant plants infected with isolate R-10 of *Fusarium oxysporum*. The arrows indicated symptoms caused by the pathogen. A; Comparison of symptoms of control and mutant plants infected with R-10 of *Fusarium oxysporum*, B; Vertical section of control plant, and C; Vertical section of mutant plant infected with R-10 of *Fusarium oxysporum*.

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